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Review paper

Application of microfluidic technology based on surface-enhanced Raman scattering in cancer biomarker detection: A review

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ABSTRACT

With the continuous discovery and research of predictive cancer-related biomarkers, liquid biopsy shows great potential in cancer diagnosis. Surface-enhanced Raman scattering (SERS) and microfluidic technology have received much attention among the various cancer biomarker detection methods. The former has ultrahigh detection sensitivity and can provide a unique fingerprint. In contrast, the latter has the characteristics of miniaturization and integration, which can realize accurate control of the detection samples and high-throughput detection through design. Both have the potential for point-of-care testing (POCT), and their combination (lab-on-a-chip SERS (LoC-SERS)) shows good compatibility. In this paper, the basic situation of circulating proteins, circulating tumor cells, exosomes, circulating tumor DNA (ctDNA), and microRNA (miRNA) in the diagnosis of various cancers is reviewed, and the detection research of these biomarkers by the LoC-SERS platform in recent years is described in detail. At the same time, the challenges and future development of the platform are discussed at the end of the review. Summarizing the current technology is expected to provide a reference for scholars engaged in related work and interested in this field.

1. Introduction

Globally, cancer remains a significant medical challenge, despite continuous progress in the development of medical science and technology. According to the GLOBOCAN 2020 of the International Agency for Research on Cancer (IARC), 19.3 million new cancer cases and nearly 10 million cancer deaths were recorded worldwide in 2020 alone [1]. The incidence and mortality rate of cancer still show an increasing trend year by year [2].

Compared with primary tumors, secondary tumors (metastases) are the chief culprit of cancer patient death. Therefore, to improve the survival rate of patients, it is an ideal and practical choice to diagnose presymptomatic cases in a timely manner through routine screening programs [3]. A commonly used method for cancer diagnosis is tissue biopsy, which is also considered as the gold standard, but the disadvantages of tissue biopsy are apparent. For example, invasive sampling often increases the discomfort of patients, and at the same time, there is a potential risk of complications [4,5]. Moreover, it cannot capture the genetic heterogeneity within tumors and between tumor metastases, thus affecting the accuracy of the test [6,7]. Suppose there is a detection technology whose accuracy is comparable to or even higher than that of tissue biopsy, with lower risk and higher efficiency, that will be revolutionary in cancer diagnosis.

With the development of various cancer diagnosis technologies, liquid biopsy is gradually attracting attention. Unlike tissue biopsy, liquid biopsies determine physiological and pathological information by analyzing circulating targets in various body fluids (e.g., blood, saliva, and urine). This process is much less invasive than tissue biopsy for patients, and at the same time, the time cost is reduced due to the relatively simple operation [8]. In addition, liquid biopsies can analyze circulating targets released by early tumors and are essential for early cancer detection. At the same time, liquid biopsy is crucial in treating both primary and secondary tumors, as it significantly enhances interpretation capacity, which is beneficial for accurate and personalized treatment [9,10]. Finally, because liquid biopsy is milder for operating conditions and allows repeated analysis, it can monitor the development of the disease at any time [11].

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With the introduction of liquid biopsies, there has been a new wave of testing for various cancer biomarkers [12]. The unique gene expression of cancer cells makes them identifiable in tissues where surrounding cells do not express specific biomarkers [13]. Tumor-derived biomarkers (cancer biomarkers) mainly include circulating proteins, circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), microRNA (miRNA), and exosomes [14–18]. In addition, small molecular compounds and carbohydrates also have potential for cancer diagnosis [19]. Theoretically, there are many sources of biomarker detection. Nevertheless, peripheral blood is the most important, and detecting cancer biomarkers in other body fluids needs more practical verification with only a few exceptions, such as a specific Food and Drug Administration (FDA)-approved tumor marker, PCA3, which is an indicator of prostate cancer risk derived from urine [4].

The assays used to detect cancer biomarkers are mainly based on the identification and detection of corresponding proteins and nucleic acids, such as enzyme-linked immunosorbent assay (ELISA) [20,21], polymerase chain reaction (PCR) [22,23], next-generation sequencing (NGS) [24,25], and mass spectrometry (MS) [26,27], which will be further summarized in the description of various biomarker detections in this paper. These conventional techniques have shortcomings, such as complicated operation steps, time consumption, high cost, and high operator experience requirements. In addition, the complexity of the body fluid environment and the scarcity of biomarkers require methods with very high specificity and sensitivity. Therefore, some of these methods need to be appropriately replaced if used for point-of-care testing (POCT).

The microfluidic chip based on surface-enhanced Raman scattering (SERS) detection (also known as lab-on-a-chip SERS (LoC-SERS) [28,29]) shows great potential in cancer biomarker detection. Microfluidic technology has the characteristics of miniaturization and integration and can realize accurate control and high-throughput detection of samples through design of microfluidic system. SERS has ultrahigh detection sensitivity and can provide unique fingerprints. Therefore, much literature has been published to analyze biomarkers based on these two methods. More excitingly, the combination of the two technologies shows good compatibility and opens up new opportunities to provide a sensitive detection method with reproducible measurement conditions and a highly defined detection area specified by the system on a chip [30]. The combination of the two technologies can also accomplish one of the goals of POCT, that is, chip-based, miniaturized, portable, and independent system testing [31].

This paper describes the benefits of SERS and microfluidic technology, the two essential components of LoC-SERS. On this basis, the application of this combined technique to detect several common biomarkers in recent years will be reviewed, including circulating proteins, CTCs, ctDNA, miRNA, and exosomes. We will also discuss the challenges associated with LoC-SERS technology and prospects at the end of the review.

We used PubMed and Web of Science to search for the following characters independently or in combination: SERS, microfluidic technology, cancer biomarkers, cancer-related proteins, circulating tumor cells, exosomes, miRNA, ctDNA, and obtained relevant documents, among which the latest cases of exploratory research on the combined application of SERS (or scattering) and microfluidic technology in the detection of various cancer biomarkers from the last ten years are emphatically provided for readers, as shown in Fig. 1. What needs to be put forward is that we have not included all the papers in the time range, and there is no prejudice when selecting documents. By summarizing this technology at present, it is expected to provide references for scholars engaged in related work and interested in this field.

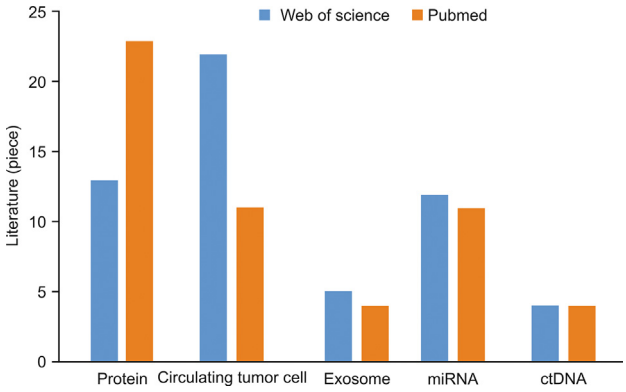


Fig. 1. The number of literature about microfluidic chips based on surface-enhanced Raman scattering (SERS) to detect various cancer biomarkers from the last ten years. Data was acquired from the Web of Science and PubMed Central. miRNA: microRNAs; ctDNA: circulating tumor DNA.

2. LoC-SERS

2.1. Brief introduction to a microfluidic chip

The origins of microfluidics development date back to the 1990s [32]. The microfluidic chip, also known as lab-on-a-chip [33], is the main platform for realizing microfluidic technology. Therefore, the microfluidic technology mentioned in this paper is mainly introduced based on various microfluidic chip platforms. Since the concept of microfluidics was put forward, its research in many fields has been well developed, and its application in biomedicine is more extensive, such as preparing analytical samples, enriching rare analytes, and on-site diagnosis [34]. The most significant advantage of microfluidic platforms lies in their miniaturization and integration.

With the development of microfluidics, the integrated components of microdevices and microsystems are becoming increasingly abundant, such as valves, pumps, microchannels, mixers, reactors, separators, and sensors, thus realizing an increasing number of functions, including the transmission, separation, mixing, reaction, and detection of tiny amounts of fluids [35]. Various materials, such as glass, silicon wafers, and paper (including cellulose fiber and nitrocellulose fiber), can be utilized as substrate elements of microfluidic devices. In addition, polymers are incredibly widely applied in preparing microfluidic devices such as polydimethylsiloxane (PDMS) [36–38]. The preparation methods of microfluidic platforms mainly involve photolithography and etching. For instance, in the photolithography procedure, the prepared PDMS liquid prepolymer can be cast on a photoresist template and thermally cured at a specific temperature. Then, the microfluidic device can be peeled off from the template due to its low surface tension [39].

According to the different driving modes, microfluidic chips can generally be divided into two types, namely, mechanical driving mode and nonmechanical driving mode. As the name implies, the former requires external mechanical moving parts to exert driving force (such as positive pressure and negative pressure exerted by injection pump or extraction pump, centrifugal force, pneumatic driving, electrostatic driving, and electromagnetic driving, etc.) to promote the flow of fluid in microfluidic chips, which is also called active driving. In contrast, the latter does not use mechanical moving parts but generates driving force through the interaction between fluid and microchannel at the microscale (such as gravity, capillary, and surface tension), or the chip itself can generate pressure to drive the sample through design (such as pressure driving, vacuum driving, and osmotic driving), which is also called

passive driving. Table 1 [40–63] shows several common microfluidic chip types, characteristics, and preparation materials based on two driving modes. It can be concluded that microfluidic chips based on the mechanical driving mode are more controllable and stable because of the participation of external devices and can realize more chip functions. Nevertheless, at the same time, the operation is more complicated, which is not conducive to use at anytime and anywhere. Comparatively speaking, microfluidic chips based on the nonmechanical driving mode are easier to manufacture and use, and the manufacturing cost is lower, so they have more significant POCT potential. At the same time, they face greater challenges for the continuity and stability of fluid flow without the assistance of other equipment.

As mentioned earlier, liquid biopsy's knotty problem is that biomarker levels are often deficient, and biological samples are very complex. In this case, microfluidic technology offers several advantages. First, a tiny amount of liquid flows in the laminar flow in the microfluidic platform, which can be precisely controlled by adjusting the flow rate. In a relatively controlled time and space, this feature enables biomarkers (such as cancer cells) to be delivered effectively and accurately, and the microfluidic chip, a relatively complete and closed system, can effectively save space and reduce pollution [64]. Second, due to the structural characteristics of microfluidics, the consumption of samples and reagents can be minimized with the platform, improving sensitivity [65]. Furthermore, compared to traditional laboratory methods, this platform's high level of integration significantly reduces analysis costs and time [38]. Finally, advances in microfluidics have also contributed to the emergence and development of the POCT concept [66].

POCT is a method that can analyze and obtain test results rapidly at the sampling site. One of its primary characteristics is that it does not depend on complicated processing procedures in laboratory tests [67,68]. Based on this feature, realizing easy-to-use diagnostic tests has become one of the main challenges POCT biosensors face in medical applications [69]. Microfluidic technology, because of its miniaturization and integration, can better meet the requirements

Table 1
Comparison of common microfluidic chips based on mechanical and nonmechanical driving modes.

Driving type	Driving method	Key driving element	Microfluidic chip materials	Characteristic	Refs.
Mechanical drive	Pressure driven	Syringe pressure pump	Glass and PDMS	Stable	[40]
	Pressure driven	Portable plug-and-play syringe pump	PDMS, silicon film, and PET film	Self-sufficient and portable	[41]
	Pneumatic driven	Thermo-pneumatic micropump	Glass and PDMS	Low-cost, simple, and moderate flow rate	[42]
	Pneumatic driven	Oil-hydraulic micropump	Glass and PDMS	High pumping rate and can withstand a great back pressure	[43]
	Pneumatic drive	Pneumatic solo diffuser-nozzle micropump	PMMA and TPU	Compact architecture and convenient	[44]
	Piezoelectric driven	Three piezoelectric actuators	Glass and PDMS	Low-cost and high-precision	[45]
	Piezoelectric driven	Optopiezoelectric composite	Optopiezoelectric composite and PDMS	Small in size and quantity of driving units	[46]
	Reciprocating driven	Reciprocating elastomeric micropump	No. 1 borosilicate coverslip and PDMS	Fast and flexible	[47]
	Centrifugal force	Modularized centrifugal microfluidic chip	PMMA	High in freedom and accuracy of chip control	[48]
	Centrifugal force	Centrifugal microfluidic chip	Glass and PDMS	Ultrafast, label-free, and efficient	[49]
	Electrostatic driven	Electrostatic bending actuators	Si	High pumping rate and can withstand a great back pressure	[50]
	Electromagnetic driven	Electromagnetic coils	Glass and PDMS	Low-cost and high-efficiency	[51]
Nonmechanical drive	Pressure driven	Pressure-driven PDMS pump	PDMS	Multifunctional, independent, flexible, simple, and low-cost	[52]
	Pressure driven	Medium storage container	PDMS	High controllability and flexibility	[53]
	Gravity driven	Gravity-driven microfluidic chip	PDMS	Fast, simple, recyclable, and POCT potential	[54]
	Gravity driven	Gravity-driven cyclic microfluidic chip	PDMS	Stable, simple, and recyclable	[55]
	Capillary force	Whatman chromatography paper	Paper and PDMS	Low-cost and reproducible	[56]
	Capillary force	Microchannel	PMMA	Cheap and portable	[57]
	Surface tension	Microchannel and Peltier device	Glass and PDMS	Convenient and POCT potential	[58]
	Surface tension	Two-well hanging droplet device	Polystyrene sheet	Enables dynamic culture and analysis of tissues	[59]
	Vacuum driven	Parylene C-coated PDMS microfluidic chip	PDMS	Long maintenance time of vacuum driving power and POCT potential	[60]
	Vacuum driven	Disposable vacuum module	Glass and PDMS	Compact, simple structure, and east-to-attach with the microfluidic device	[61]
	Osmotic driven	Semipermeable membrane	PMMA	User-friendly and accurate speed control	[62]
	Osmotic driven	Semipermeable membrane	PDMS	Low cost, small size, and stable flow rate	[63]

PDMS: polydimethylsiloxane; PET: polyethylene terephthalate; PMMA: polymethyl methacrylate; TPU: thermoplastic polyurethane; POCT: point-of-care testing.

of POCT, so it is considered as one of the most promising solutions [70]. The compatibility between the microfluidic platform and POCT also reduces the operation requirements of detection and is of great significance for popularization and use in areas with limited resources [71].

2.2. Brief introduction to SERS

Detection is essential for microfluidic analysis. Common microfluidic detection methods include laser-induced fluorescence spectroscopy [72,73], ultraviolet-visible spectroscopy [74], MS [75,76], electrochemical analysis [77,78], etc.. Among all kinds of optical detection methods, Raman spectroscopy, as a unique fingerprint spectrum, has attracted attention in recent years in cancer biomarker research by combining it with microfluidic technology.

Raman scattering was first discovered by Indian scientist Raman in 1928 [79]. The principle of the Raman spectrum belongs to the scattering spectrum. The interaction between light and molecules or atoms will produce two types of scattering: Rayleigh and Raman scattering. The former belongs to elastic scattering and plays a dominant role in scattering, meaning that the incident light does not change in frequency after being scattered. The latter belongs to inelastic scattering; after the incident light is scattered, some energy is gained or lost, resulting in a frequency change, which only accounts for a small part of the scattering [80]. Compared with other spectroscopic techniques based on molecular vibration (such as near-infrared spectroscopy), Raman spectroscopy can not only interpret the chemical information and structural information of the analyte through the spectral shift, representing the chemical fingerprint of the substance but also be free from the interference of water in the analyte, which provides certain advantages for expanding the analytical range of Raman scattering [81,82]. The most prominent problem of Raman scattering is that its scattering signal is feeble, only one-millionth of the incident light intensity [83]. Meanwhile, compared with Rayleigh scattering, Raman scattering accounts for a small part, and its light scattering cross section (i.e., the area of the incident beam where incident photons are effectively converted into emitted Raman photons) is several orders of magnitude weaker [84]. These reasons explain why Raman scattering has developed slowly since its discovery. Thus, given the above characteristics, Raman scattering is not sensitive enough for many practical applications, such as biomarkers.

The application of Raman spectroscopy developed rapidly after the SERS phenomenon was discovered. In 1974, Fleischmann et al. [85] found that the pyridine monolayer showed stronger Raman scattering intensity when adsorbed on a rough silver electrode. With the development of research, the enhancement mechanism is mainly attributed to electromagnetic enhancement and chemical enhancement [84,86]. For electromagnetic enhancement, surface plasmon resonance caused by the collective oscillation of free electrons excited by photons confines the electromagnetic field to the substrate surface, and the local electromagnetic field enhanced by the substrate surface amplifies the Raman signal of molecules adsorbed on the substrate [87], as shown in Fig. 2A. The enhancement of the scattered photon intensity is proportional to the fourth power of the electromagnetic field, which explains the amplification of the Raman signal of molecules near the plasma substrate [88,89]. It preferentially appears in gaps, tips, and other corner parts (i.e., “hot spots”) of noble metals with nanoproperties (e.g., silver, gold, and copper) [90]. In addition, when the analyte forms a chemical bond with the metal surface, electrons transfer from the metal to the surface and vice versa. This changes the molecule's effective polarizability and produces an enhancement effect termed chemical enhancement [91]. The chemical enhancement effect is much weaker than the electromagnetic enhancement effect. Its enhancement factor (EF) is only 10^2–10^3, while that of the former can reach 10^{10}–10^{11} [92,93], as shown in Fig. 2B. In addition, the electromagnetic enhancement effect usually occurs in any Raman-active molecule indiscriminately, while the chemical enhancement effect possesses molecular specificity [94]. Therefore, most SERS-based biosensors have been developed by exploiting the plasma properties of nanostructures.

A substrate based on noble metal rough nanostructures is the key to realizing Raman signal enhancement. However, some new SERS substrate materials have also been developed, such as two-dimensional (2D) layered nanostructures (graphene [95,96], hexagonal boron nitride [97,98], black phosphorus [99,100], MXenes [101,102], etc.), and semiconductor materials (NiO [103,104], TiO_2 [105,106], ZnO [107,108], GaP [109,110], etc.), but the Raman signal enhancement effect of most new materials is still not as good as that of traditional noble metals, so they are usually used in combination with noble metals. SERS substrates can be divided into two forms: nanosol and solid substrates. The former is thoroughly mixed with the sample before Raman detection, and common preparation methods include chemical reduction growth, light wave irradiation, microwave irradiation, catalytic reaction, etc. In the latter, nanomaterials are prepared into solid substrates by filtration, spin coating, electrodeposition, chemical vapor deposition, and other technologies. Then, Raman detection is carried out after the sample is combined with the solid substrate. Table 2 [111–127] shows SERS nanosols and solid substrates prepared by

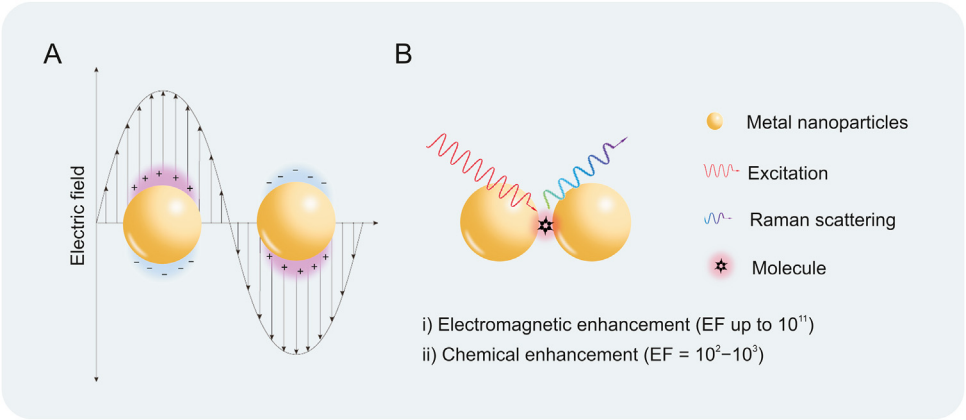


Fig. 2. Diagram of the basic mechanism of surface-enhanced Raman scattering (SERS). (A) Illustration of the collective oscillation of free electrons on the surface of metal nanoparticles. (B) Schematic diagram of electromagnetic enhancement and chemical enhancement of SERS. EF: enhancement factor.

several commonly used methods. It can be seen from the table that both forms can enhance the Raman signal well, but their advantages are also different. Nanosol emphasizes simple preparation and rapid detection, while solid substrate emphasizes repeatability of the detection signal and stability of the substrate.

SERS has become a promising method to detect various biomarkers due to its high sensitivity, multiple analysis, and insensitivity to photobleaching [128]. There are two main types of SERS biosensors: label-based and label-free. Based on label-free SERS detection, analyte information can be obtained directly. Nevertheless, it is required that the analyte has SERS activity. At the same time, the influence of the detection specificity of other interfering substances in the analysis environment should be avoided. Detection based on the SERS label cannot provide specific analyte information directly. However, the SERS signal obtained by the Raman reporters can replace the measurement of the analyte, thus enhancing the interface specificity of detection [129,130]. Whether based on Raman labeling or not, the SERS signal obtained is relatively isolated, which provides suitable conditions for subsequent spectral processing [131].

2.3. Microfluidics and SERS: complementary advantage

LoC-SERS technology can enhance the advantages of microfluidics and SERS, thus showing compatibility [132]. First, with the introduction of microfluidic technology, large-scale processes prone to human errors in the laboratory can now be carried out automatically and repeatedly in nanoliter volumes. At the same time, SERS can meet the needs of microfluidic platforms to detect small volumes [133]. In addition, water interference can be effectively avoided by the SERS method, making it more suitable for liquid biopsies, and the relatively closed system of a microfluidic chip can better solve the problem that the sample molecules on the SERS substrate surface are easily contaminated.

Moreover, the single-component analysis of biomarkers may not fully explain the disease and provide enough accuracy for decision-making [134]. It is, therefore, common practice in clinical and biomedical research to combine various biomarkers to improve overall diagnostic performance [135]. The LoC-SERS platform makes it possible for multiple accurate diagnoses of biomarkers. On one hand, high-throughput detection of biomarkers can be realized through chip unit design [136]; on the other hand, the ultrahigh sensitivity of SERS facilitates the detection of complex samples.

Finally, the two technologies have strong POCT potential, and their combined use makes detection more efficient and convenient. SERS technology can be flexibly combined with a microfluidic platform [137]. A notable trend in portable Raman instruments today is their smaller size. However, their performance (signal-to-noise ratio for the same acquisition time and resolution) is improving, so people can take the spectrometer to the sample at any time [138], making POCT possible. Some studies even use smartphones as miniaturized Raman spectrum analyzers [139,140], thus achieving revolutionary advantages such as a friendly man-machine interface and fast measurement time [141].

Table 2
Overview of surface-enhanced Raman scattering (SERS) nanosols and solid substrates prepared by commonly used methods.

Substrate type	Nanoparticles	Method of preparation	Shape	Size	Characteristic	Refs.
Nanosol	Au	Chemical reduction	Nanoflower	150 nm	Simple, rapid, and sensitive	[111]
	Ag	Chemical reduction	Nanotriangle	10–70 nm	Stable	[112]
	Ag	Chemical reduction	Spherical	40 nm	Simple, selective, and sensitive	[113]
	Ag	Chemical reduction and microwave irradiation	Nanorod	Diameter of about 12 nm and a length of about 15–40 nm	Simple, selective, and sensitive	[114]
	Au	Light-wave procedure	Spherical	20 nm	Simple, selective, and sensitive	[115]
	Ag	Light-wave procedure	Spherical	13 nm	Sensitive and selective	[116]
	Ag	Light-wave procedure and catalysis	Spherical	10 nm	Sensitive	[117]
Solid substrate	Au	Chemical reduction and filtration	Nanostar	236 ± 11 nm	Simple, low-cost, and reproducible	[118]
	Ag	Spin-coating followed by thermal reduction	Spherical	100 nm	Simple, low-cost, and reproducible	[119]
	Au	Reactive ion etching and ion sputtering deposition	Nanocap	30 nm	Sensitive and uniform	[120]
	Ag-Au nanocomposite	Electrodeposition and galvanic replacement	Spherical	110 nm	Sensitive, stable, and reproducible	[121]
	Graphene/Au nanodots	Chemical vapor deposition and focused ion beam technique	Nanodot	95 ± 2 nm	Sensitive	[122]
	Graphene/Ag-Cu nanoparticle hybrid system	Chemical vapor deposition	Island shape	30 nm	Sensitive and reproducible	[123]
	Ag	Filtration and magnetron sputtering	Spherical	12–31 nm	Homogenous and stable	[124]
	Au	Reactive ion-etching process and magnetron sputtering	Spherical	50 nm	Sensitive and homogeneous	[125]
	Ag	Magnetron sputtering	–	31.9 ± 8.4 nm	Sensitive and low-cost	[126]
	Fe_3O_4-Au core-satellite nanocomposites and Fe_3O_4@Au core-shell nanocomposites	Thermal decomposition, seed deposition method (core-satellite), and seed-mediated growth method (core-shell)	Hexahedral	70 nm (core-satellite), 83 nm (core-shell)	Reproducible, recoverable, and reusable	[127]

Given these advantages, SERS technology and the microfluidic platform certainly make for a win-win combination that will make greater contributions to cancer biomarker detection.

3. LoC-SERS used to detect circulating proteins

3.1. Circulating proteins in liquid biopsy

Circulating protein biomarkers are representative of diagnosing early cancers [142]. Proteins are more diverse and carry more information than nucleic acid molecules such as DNA and RNA. Among all kinds of biomolecular information sources used to characterize and diagnose cancer, protein biomarkers have the most extended history in clinical practice [14]. Almost all cancer biomarkers approved by the FDA are proteins related to various behaviors of tumor cells, such as proliferation, invasion, immune regulation, invasion, angiogenesis, carcinogenesis signal transmission, and metastasis [143,144]. Since Gold and Freedman identified carcinoembryonic antigen (CEA) in colon tissue extract in the 1970s, cancer antigens have been studied as biomarkers for early cancer detection [145], for instance, prostate-specific antigen (PSA) for prostate cancer [146,147] and various carbohydrate antigens such as CA19-9 for pancreatic cancer [148], CA-125 for ovarian cancer [149], and CA-15.3 for breast cancer [150]. However, it is worth noting that although these circulating proteins offer a new way to track patients with known diseases as biomarkers for cancer diagnosis, none of them has been approved for screening purposes due to their sensitivity and specificity [151]. Developing a more sensitive method for combined detection will be helpful. For example, measuring a group of protein biomarkers is usually required simultaneously to avoid false results [152,153].

3.2. Analysis method of circulating proteins

Usually, when designing and manufacturing a biosensor, good sensitivity, specificity, and accuracy are the factors that must be considered [154]. Currently, most methods for detecting and discovering protein biomarkers are primarily based on antibody-based assays, and ELISA is recognized as a standard technique [155–157]. In addition, MS, microarray, and molecular imprinting are also used to detect protein markers. Table 3 [20,21,26,27,158–162] presents the use of the above methods for the detection of circulating proteins in cancer. With increased assay requirements, various assays are moving toward high sensitivity. Whereas ease of operation and low cost are also necessary for protein POCT equipment [163], implementing many methods relies on complex instrumentation or operational steps, such as expensive instruments, highly skilled personnel, and lengthy analysis times when using MS. The microarray method can realize multiple measurements of proteins. Nevertheless, the low availability of suitable capture probes is the biggest obstacle when microarrays are used in large-scale protein group research [164]. In recent years, sandwich immunoassays have become a popular strategy for cancer biomarker detection, which can capture the target protein analyte by functionalizing the sensor with an aptamer or antibody [144]. However, even standard methods such as ELISA are constantly improved or combined with other technologies to further enhance their sensitivity and specificity. For example, Zhou et al. [165] made a stable gold nanoparticle layer for detecting CEA in plasma by chemical reduction on a commercial ELISA plate. The improved ELISA method showed better sensitivity and stability for proteins. Woodbury et al. [166] combined microarray-ELISA with the signal amplification of tyramide deposition and achieved ultrasensitive detection of hepatocyte growth factor at the sub pg/mL level. Similar to ELISA immunoassays, SERS immunoassays are usually developed by sandwich structures [167–169], but SERS immunoassays possess higher sensitivity, lower interference, and multisensor potential than ELISA [170]. Combined with the microfluidic platform, the detection process of SERS is more controllable and convenient, so it also provides a novel idea for detecting protein biomarkers.

3.3. Applications of LoC-SERS in circulating protein analysis

When using the sandwich immune complex strategy to detect proteins, a specific antibody is initially bound to the substrate as a capture antibody. Meanwhile, as a detection antibody, the antibody is combined with metal nanoparticles, and Raman reporters label the metal nanoparticles to form SERS probes. When there is a target protein in the system, it can specifically combine with the detection antibody on the SERS probe and the capture antibody on the substrate, forming a basic “sandwich” immune complex structure. The formation of this complex is helpful to make the reporters on the SERS probe enter the hot spots and then generate a strong Raman signal. For instance, Li et al. [171] detected IgG in serum using a “molecularly imprinted polymer-target molecule-magnetic SERS probe” sandwich complex. Enriching the probes and enhancing the hot spots were achieved under an external magnetic field.

The introduction of microfluidics provides advantages for the quantification of protein biomarkers. The microfluidic system channel's high surface area/volume ratio allows for easier and more rapid formation of antigen-antibody immune complexes. In

Table 3
Methods utilized for the detection of circulating proteins.

Related cancer types	Target	Detection method	Limit of detection	Linear detection range (ng/mL)	Refs.
Breast cancer	OPN	ELISA	13.9 pg/mL	0.078–10	[20]
Neuroendocrine neoplasms	CgA	ELISA	2.4 ng/mL (limit of blank)	0–870	[21]
Liver cancer	AFP	MS	0.15 ng/mL	0.5–20	[26]
Colorectal cancer	CEA	MS	0.6×10^{-3} ng/mL	0.05–100	[27]
Prostatic cancer	PSA	SIL/MRM-MS	0.28 fmol	5–200	[158]
Hepatocellular carcinoma	AFP	Fluorescence protein microarrays	–	0.03125–80	[159]
Prostatic cancer	F-PSA and c-PSA	Dual-color quantum dots nanobeads-based suspension microarray	22 and 45 pg/mL	0.64–80.0 and 0.2–50.0	[160]
Pancreatic cancer	CEA	Molecular imprinting	0.5 ng/mL	–	[161]
Colorectal cancer	CEA	Molecular imprinting	–	2.5–250	[162]

–: no data. OPN: osteopontin; CgA: chromogranin A; ELISA: enzyme-linked immunosorbent assay; AFP: alpha-fetoprotein; MS: mass spectrometry; CEA: carcinoembryonic antigen; PSA: prostate-specific antigen; SIL/MRM-MS: stable isotope labeling-multiple reaction monitoring mass spectrometry; F-PSA: free form of PSA protein; c-PSA: α1-antichymotrypsin-complexed PSA.

addition, microfluidic devices reduce the nonspecific adsorption of SERS nanoprobes by a continuous flow of liquid in the microchannel or by external action (such as an external magnetic field), thereby increasing the sensitivity, accuracy, and specificity of the assay [128,172]. As shown in Fig. 3, there are mainly two binding forms of sandwich immune complexes in microfluidic devices, namely, those based on a nonfixed SERS substrate and those based on a fixed SERS substrate. In the former, capture antibodies of the substrate are mixed with the probes and antigens through microchannels or microchambers to form immune complexes, which are then aggregated at a specific location on the chip by external magnetic fields and other methods for SERS detection. The latter is to fix the substrates (such as metal nanoparticles) containing the capture antibodies in channels or chambers of a microfluidic chip. When the probes and antigens pass through the regions where the substrates are located, they form immune complexes, and then SERS detection is facilitated.

3.3.1. Nonfixed SERS substrate used in LoC-SERS

Due to the external magnetic field, the formed immune complex can be effectively aggregated to form many hot spots. They can also be separated from other substances that do not form immune complexes. Ahi et al. designed a microfluidic chip driven by capillaries, which consists of four chambers, as shown in Fig. 4A [173]. As capture probes, magnetic metal-organic framework nanoparticles were used to capture antigens and then combined with antibody-modified and 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) molecular-labeled gold nanoparticles in a microfluidic chip. A simple magnet moved them between chambers, and SERS was detected in the last chamber. The intersection of the microchannel and the chamber forms a capillary burst valve that enables the pipette to continuously load samples and reagents into each chamber through separate inlet and outlet holes without spilling the liquid into the adjacent chambers or mixing the solutions. The team successfully detected human chorionic gonadotropin (hCG) protein in the urine, a stimulant of male athletes, and a substance related to many cancers [174,175]. The developed method is fast and reliable and can realize the combination, enrichment, and detection of immune complexes under the action of an external magnetic field. The study also showed that the results obtained using this method were consistent with those obtained by conventional ELISA.

Likewise, droplet microfluidics combined with SERS has also been reported to be applied to protein biomarker determination, eliminating the need for repeated washing steps and making detection more convenient than traditional nonfixed SERS substrates. Gao et al. designed a SERS-based droplet microsensor consisting of five parts for the detection of PSA, as shown in Fig. 4B [176]. Malachite green isothiocyanate-labeled gold nanoparticles and magnetic beads were modified with PSA antibody. When PSA appeared in the system, the antigen-antibody magnetic immune complex was formed through the complete mixing of curved channels, and droplets were formed under the action of the oil phase. When they pass through the magnetic field, the droplets are divided into two smaller parts, the immune complex droplets are adsorbed in the magnetic field, and the unbound gold nanotag droplets are used for SERS detection. In short, when there are more PSAs in the system, fewer unbound gold nanotags will be used for quantitative analysis. The clinical threshold for PSA detection by qualified sensors should be at least 4 ng/mL, whereas the above method's limit of detection (LOD) is lower than 0.1 ng/mL.

3.3.2. Fixed SERS substrate used in LoC-SERS

As mentioned above, multiple protein markers are usually selected for simultaneous detection to improve the specificity, which can be realized by modifying various antibodies on fixed SERS substrates. Zheng et al. designed a spatially separated detection biosensor using multiple channels in a microfluidic chip, as shown in Fig. 4C [177]. CA153, CA125, and CEA were selected as biomarker models of breast cancer, and their detection with high sensitivity and specificity in human serum was realized. In this work, silver nanoparticles were first fixed on a glass sheet by electrostatic adsorption to form a SERS-active substrate, and then the capture antibody, sample, and immune SERS probe (4-mercaptobenzoic acid (4-MBA), DTNB, and 2-naphthalenethiol were used as reporter molecules, respectively) were injected into the channel in turn. If the above target antigens are present in the sample, immune sandwich complexes will be formed, and the concentrations of the antigens correlate with the SERS signal. By using a variety of antibodies and channels, this microfluidic chip based on SERS can realize high-throughput detection of samples. Finally, the LODs of CA153, CA125, and CEA in serum reached 0.01 U/mL, 0.01 U/mL, and 1 pg/mL, respectively, lower than the critical value of clinical diagnosis.

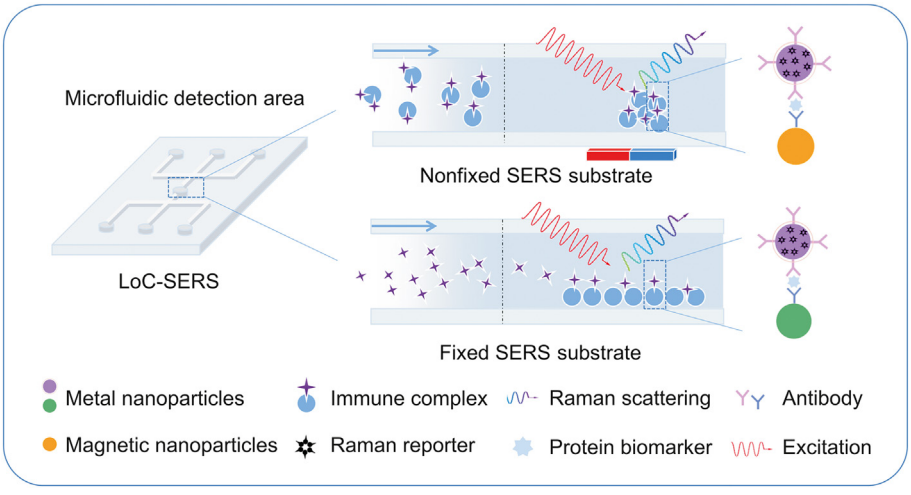


Fig. 3. Nonfixed and fixed substrates used in microfluidic chips based on surface-enhanced Raman scattering (lab-on-a-chip SERS (LoC-SERS)).

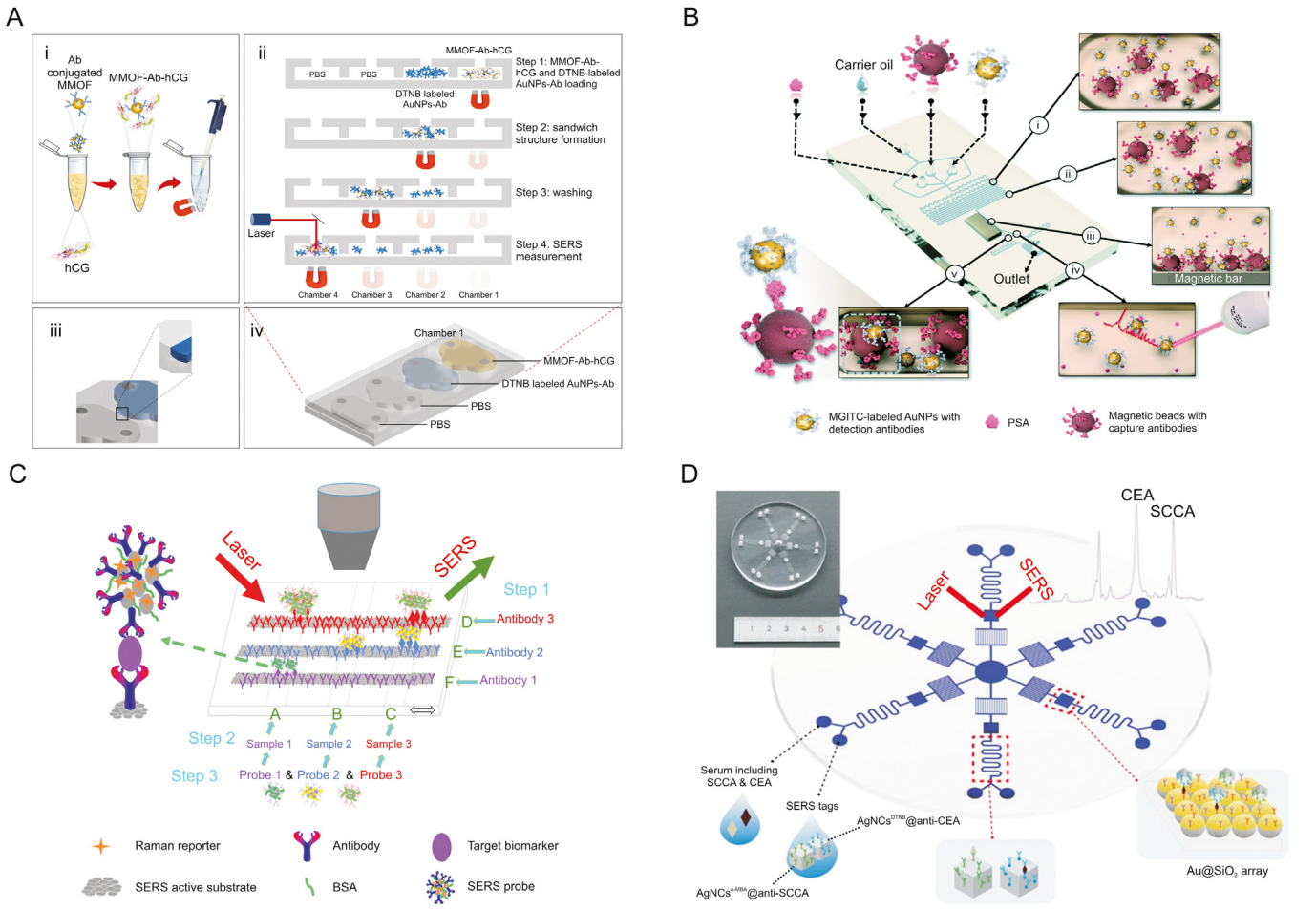


Fig. 4. Microfluidic chips based on surface-enhanced Raman scattering (lab-on-a-chip SERS (LoC-SERS)) for detection of circulating proteins. (A) Schematic illustration of the capillary-driven microfluidic chip for SERS-based human chorionic gonadotropin (hCG) detection [173]. Immunomagnetic separation of hCG from urine samples (i), the step-by-step sandwich immunoassay procedure (ii), burst valve between chambers (iii), and microfluidic chip loaded with different solutions (iv). (B) Schematic illustration of the SERS-based microdroplet sensor for wash-free magnetic immunoassay [176]: (i) droplet generation and reagent mixing, (ii) formation of magnetic immunocomplexes, (iii) magnetic separation of immunocomplexes, (iv) generation of larger droplets containing the supernatant for SERS detection, and (v) generation of smaller droplets containing magnetic immunocomplexes. (C) Schematic illustration of the metal-enhanced three-dimensional (3D) SERS barcode microfluidic biosensor for high throughput detection of multiple breast cancer biomarkers [177]. (D) Schematic illustration of the microfluidic biosensor with Au@SiO_2 array-based highly SERS-active substrates [181]. MMOF: metal-organic framework nanoparticles; Ab: antibody; PBS: phosphate-buffered saline; DTNB: 5,5′-dithiobis-(2-nitrobenzoic acid); AuNPs: gold nanoparticles; MGITC: malachite green isothiocyanate; PSA: prostate-specific antigen; BSA: bovine serum albumin; AgNC: Ag nanocubes; 4-MBA: 4-mercaptobenzoic acid; SCCA: squamous cell carcinoma antigen; CEA: carcinoembryonic antigen. Reprinted from Refs. [173,176,177,181] with permission.

Another advantage of a fixed SERS substrate is that it can provide stable and uniform SERS hot spots for analyte detection because the size, shape, and ordered arrangement of the SERS substrates often affect the performance of the SERS signal, such as reproducibility and sensitivity [178–180]. Gu et al. used a microfluidic chip with a high-activity SERS substrate based on a Au@SiO_2 array, as shown in Fig. 4D [181], to determine biomarkers related to cervical cancer, namely, squamous cell carcinoma antigen (SCCA) and CEA. In this study, a silica microsphere array modified by gold nanoparticles was coupled with an antibody to form a capture substrate, which was combined in the detection chamber of the microfluidic platform. In addition to high SERS activity ($EF = 8.21 \times 10^6$), the ordered structure of the substrate also makes the SERS signal homogeneous. Five different batches of chips were tested on the same serum samples, and the coefficients of variation of the SERS intensity of two kinds of reporter molecules (4-MBA and DTNB) were 5.58% and 5.42% under Raman shifts of 1594 cm^{-1} and 1330 cm^{-1}, respectively. In addition, the LODs of SCCA and CEA in human serum obtained by this platform were as low as 0.45 and 0.36 pg/mL, respectively. The results were consistent with those of ELISA.

4. LoC-SERS used to detect circulating tumor cells

4.1. Circulating tumor cells in liquid biopsy

Cells are not only the most basic structural and functional units of organisms, but the signal transduction between cells is also the basis for realizing various biological functions and life activities, such as apoptosis, proliferation, differentiation, changes in cell components or their microenvironment, and is closely related to the occurrence and development of diseases [182]. Thus, cells are also essential biomarkers for disease diagnosis [183]. Typical cell diagnosis is represented by the diagnosis of CTCs, which has received great attention in the landmark analytes of real-time liquid biopsy technology. It was reported in the literature in 2021 that more than 270 clinical trials based on CTCs had already been registered [184].

CTCs are tumor cells exfoliated from a primary tumor, which then infiltrate into the blood and lymphatic vessels and transfer to distant tissues via circulation [185]. Analysis of the number or surface molecules of CTCs is especially important for the diagnosis and treatment of various cancers. For example, the system for CTC

detection based on clinical validation has been approved by the FDA to monitor the disease progress and treatment response of patients with metastatic breast cancer, prostate cancer, and colorectal cancer [186–189]. It should be noted that there are only 1–10 CTCs per milliliter of whole blood, compared with millions of leukocytes and billions of red blood cells [154]. Therefore, the biggest challenge for cell diagnosis is accurately capturing and characterizing these rare cells from samples with complex backgrounds.

4.2. Analysis methods of circulating tumor cells

Most current techniques for capturing CTCs are based on one or more characteristics to distinguish them from normal blood cells. For instance, methods based on physical properties (size, density, charge, deformation, etc.) of CTCs, such as density gradient centrifugation [190], microfiltration [191], dielectrophoresis [192,193], and cell deformation sorting technology [194], are often used. Enrichment methods based on physical properties do not rely on special biomarker identification, which costs less and is relatively simple to operate. Nevertheless, they lack specificity, which may affect the accuracy of detection. For example, using cell size alone may lead to false-negative results due to ignoring smaller tumor cells. Therefore, other technologies can be combined to compensate for this deficiency. The cell separation system proposed by Hvichia et al. [195] is enriched based on cell size and deformability, and the average cell capture rate of the device is between 42% and 70%.

Biological-based separation methods need to capture the complex combination between the recognition ligand and the target (such as antibody, peptide, and aptamer) on the medium and depend on the difference in specific cell membrane antigen expression levels between tumor cells and blood cells [196], mainly including immunomagnetic separation [197,198] and nanostructure-based separation [199,200]. Cell separation and enrichment can be realized in positive and negative selection. In the former, CTCs are separated or selected from the sample, often represented by epithelial cell adhesion molecule (EpCAM); in the latter, blood cells are removed from the sample, often represented by CD45 expressed in hematopoietic cells [201,202]. In positive selection, some CTCs may underexpress or not express EpCAM due to cellular heterogeneity. Therefore, only using EpCAM as a marker to distinguish different cells may lead to false-negative results [203,204]. In recent years, other markers, such as folate receptor and sialoglycoprotein receptor (ASGPR), have also been used to identify and capture CTCs to increase their specificity [196,205,206]. Zhu et al. [207] developed a microfluidic synergistic chip on which anti-ASGPR and anti-EpCAM were modified in parallel for simultaneous capture, and the capture rate of heterogeneous circulating hepatocellular carcinoma cells in whole blood was over 85%. To reduce the effect of leukocytes on the purity of CTCs, Park et al. [208] combined physical and chemical methods; CTCs were captured using anti-EpCAM conjugated microspheres and then successfully collected by their selective sedimentation in a density gradient media and microfiltration. The method was validated to effectively capture and collect two types of CTCs in blood, MCF-7 cells (human breast cancer cells) and DMS-79 cells (small cell lung cancer cells), with recovery rates of 99% and 89%, respectively.

Another critical step after CTCs have been captured is detection and characterization. Some commonly used CTC detection methods are shown in Table 4 [209–218]. Among them, fluorescent immunolabeling plays an important role in characterizing CTC protein expression [219]. However, fluorescence analysis has disadvantages: a complex dyeing and washing process, broad emission band spectra, spontaneous fluorescence interference, phototoxicity, and fluorescent probe photobleaching [220–222]. In addition, in the relatively complex biological sample environment, when fluorescence imaging is used to identify circulating tumor cells, it often lacks sensitivity and selectivity [221]. In addition, the high false-positive rate of reverse transcription PCR, the high cost of flow cytometry, and the low sensitivity of immunocytochemistry further limit the popularization and use of these methods [212]. The characteristic Raman peak is much narrower than the fluorescence emission peak. In addition, many characteristics of SERS, such as high light stability, sensitivity (as low as single-cell detection), rapid spectral response, and molecular fingerprinting, are beneficial to cell analysis [184,219,223,224]. Therefore, in many studies, SERS is an ideal choice for CTC detection [222,225–227].

Table 4
Methods utilized for the detection of circulating tumor cells.

Related cancer types	Category	Specific target	Enrichment method	Isolation method	Detection method	Limit of detection	Recovery rate	Refs.
Breast cancer and gastric cancer	Protein	EpCAM and CK19	Positive	Immunomagnetic separation	Real-time quantitative PCR	1 cell/5 mL	–	[209]
Colorectal cancer	mRNA	CEA and β_2M	Positive and negative	Immunomagnetic separation	Real-time quantitative RT-PCR	1 cell/mL	–	[210]
Breast cancer	mRNA	CK19 and ERBB2	Negative	Density gradient centrifuge	Real-time quantitative RT-PCR	–	75.0%–93.8%	[211]
Breast cancer	Protein	EpCAM and CK19	Positive	Immunomagnetic separation	Immunocytochemistry	1 cell/10^7 mononuclear cells	89%–93%	[212]
Breast cancer	Protein	CD45 and EpCAM	Positive and negative	Immunomagnetic separation	Flow cytometry	–	–	[213]
Colorectal cancer	Protein	EpCAM	Negative	Immunomagnetic separation	Flow cytometry	–	74.8% ± 9.3%	[214]
Prostate, colorectal, and ovarian cancer	Protein	EpCAM and CK7/8	–	Density gradient centrifugation or filtration	Fluorescence detection with fluorescence in situ hybridization	<1 cell/mL	94%–100%	[215]
Prostate and breast cancer	Protein	EpCAM	–	Density gradient centrifuge	Immunofluorescence microscopy	–	92%–110%	[216]
Lung cancer	Protein	EpCAM and CD45	–	Membrane filtration system	Indirect immunofluorescence	–	90%	[217]
Ovarian cancer	Protein	CK and VIM	–	Microfiltration method	Cytomorphology and immunofluorescence	–	–	[218]

–: no data. EpCAM: epithelial cell adhesion molecule; CK: cytokeratin; PCR: polymerase chain reaction; mRNA: messenger RNA; CEA: carcinoembryonic antigen; β_2M: β_2-microglobulin; RT-PCR: reverse transcription PCR; ERBB2: human epidermal growth factor receptor 2; CD45: leukocyte common antigen; VIM: vimentin.

4.3. Applications of LoC-SERS in circulating tumor cell analysis

Combined with the above cell separation and analysis techniques, although the diagnosis of CTCs can be completed, the step-by-step separation and analysis techniques may affect the number and integrity of cells in the downstream analysis process due to cell transfer and other steps [219,228], and the complicated and lengthy detection steps are not conducive to the implementation of POCT. In addition, when cells are treated based on a single separation mechanism (such as a physical principle or biological mechanism), the specificity and accuracy of the detection results may also be challenged. Thus, integrating various technologies has become a trend in detecting CTCs. Among them, microfluidic technology shows great advantages, which can separate CTCs from other blood cells according to their physical or biological characteristics [229]. On this basis, the captured tumor cells can be analyzed in situ by adopting appropriate analysis methods, thus simplifying the operation steps, reducing the loss of CTC capture, realizing rapid and accurate detection, and laying a foundation for POCT.

4.3.1. Cell separation and analysis based on biological characteristics

In cell separation based on biological characteristics, similar to fluorescence immunoassays, the SERS magnetic probe can bind to the markers on the surface of CTCs so that CTCs can be separated and enriched from the complex system by an external magnetic field and then further detected through the Raman signal in the probe. As mentioned above, if only one cell marker (such as EpCAM) is used for cell analysis, it will probably lead to false-negative results. Wilson Jr. et al. proposed a new multiple CTC detection method, and the detection platform is shown in Fig. 5A [203]. Transparent quartz slides were used to prepare the chip, which can overcome the effects of Raman background and fluorescence interference. Iron oxide-gold (IO-Au) core-shell SERS nanotags prepared in this study have two main functions: immunomagnetic separation of CTCs and detection by SERS spectroscopy in microfluidic devices. In this study, CD45 was used as a negative selection, and EpCAM, HER2, CD44, and insulin-like growth factor 1 receptor (IGF1R) were used as a positive selection; meanwhile, cell morphology and 4',6-diamidino-2-phenylindole (DAPI) staining were added. Various methods were used to improve the accuracy of the identification of CTCs so that even though CTCs were captured in the blood like a needle in a haystack, good analysis results could be obtained through the above strategies. Combining integrated IO-Au SERS nanotags with microfluidic devices increases the degree of automation and tedious sample processing, thus reducing the risk of CTC loss, which has great application prospects for the simultaneous detection of multiple CTC subpopulations in clinical samples.

4.3.2. Cell separation and analysis based on physical characteristics

The above detection is a typical biological method based on the separation, enrichment, and analysis of circulating tumor cells by using a variety of markers on the cell surface. Due to the integration of microfluidic technology, some studies have also improved the sensitivity and specificity of CTC detection by combining physical enrichment methods on microfluidic platforms. Zhang et al. developed a microfluidic platform for screening tumor cells from blood according to the size difference, as shown in Fig. 5B [230]. Under the optimal microfluidic filter gap, the cell capture rates of the breast cancer cell lines SKBR3, MCF7, and MDA-MB-231 reached 87.8%, 93.6%, and 87.8%, respectively. Residual blood cells were removed by washing, and then three different SERS aptamer nanovectors were injected into the chip, which showed good specificity and affinity for HER2, EpCAM, and EGFR. After full contact, the SERS signal was obtained for each cell. The complex spectral signal of CTCs was analyzed by the classical least square method, and then the phenotypic information of CTCs was successfully interpreted. Finally, 126 spectra of cells from four breast cancer subtypes (SKBR3, MCF7, MDA-MB-231, and Jurkat) were analyzed using partial least squares discriminant analysis (PLS-DA), and the different subtypes were accurately classified with good sensitivity and specificity.

Similarly, Gao et al. proposed a lantern-shaped bypass microfluidic channel based on size separation, which was used to separate and capture the CTCs of hepatocellular carcinoma, as shown in Fig. 5C [219]. Two SERS-aptamer nanotags were used for cell identification, and the final capture rate of CTCs reached 84%.

In addition, the appropriate frequency of the alternating electric field and the selected shape of an electric field can lead to the effective deposition of CTCs. Based on this principle, Szymborski et al. successfully separated two kinds of breast cancer cells (MCF-7 and MDA-MB-231) by combining dielectrophoresis with a microfluidic chip, and the CTCs deposited in specific areas of the chip were further identified by SERS, as shown in Fig. 5D [231]. The LOD of CTCs obtained by this method in phosphate-buffered saline (PBS) solution was 20 cells/mL. Meanwhile, it was found that the fingerprints of these two CTCs were also observed in the complex detection environment of plasma, reflecting this method's clinical potential and effectiveness.

5. LoC-SERS used to detect exosomes

5.1. Exosomes in liquid biopsy

Exosomes are cup-shaped vesicles 30–150 nm in size and are widely found in human body fluids such as blood plasma, saliva, urine, amniotic fluid, cerebrospinal fluid, ascites, and pleural fluid [232–234]. This tiny substance with a lipid bilayer was first discovered by EG Trams in 1981 and was named an exosome by Johnstone in 1987 [235]. At first, the function of exosomes was considered just to excrete the waste materials in cells and even called the "garbage bins" of cells [236]. However, in the last decade, with increasing research on exosomes, it has been discovered that this kind of vesicle, which contains a variety of bioactive substances, such as proteins, nucleic acids, and lipids, transmits signals between cells and is not only involved in regulating physiological responses but also associated with certain diseases, especially cancer, also known as the "parental cell fingerprint" [237,238]. Although all cells are known to secrete exosomes, a relatively increased number of exosomes have been reported in the body fluids of patients with various cancer types [239,240]. Tumor-derived exosomes are involved in promoting the proliferation, progression, metastasis, angiogenesis, invasion, remodeling, and drug resistance of cancer through cell-to-cell communication in the tumor microenvironment [18]. Therefore, exosomes, as an important part of cancer biomarkers, provide new ideas for cancer diagnosis and have great potential.

5.2. Analysis methods of exosomes

The reliable separation of exosomes from the complex matrix is the premise of later analysis (such as quantification and identification). However, because of their small size and low buoyant density, separating exosomes from the blood is not easy and requires much time and effort [241]. As mentioned above, exosomes come from almost all cells, including normal and tumor cells, so obtaining accurate and useful information is difficult if exosomes cannot be effectively recognized in body fluids [242]. Traditional exosome separation techniques, such as ultracentrifugation, exosome precipitation, and ultrafiltration, are widely used in research

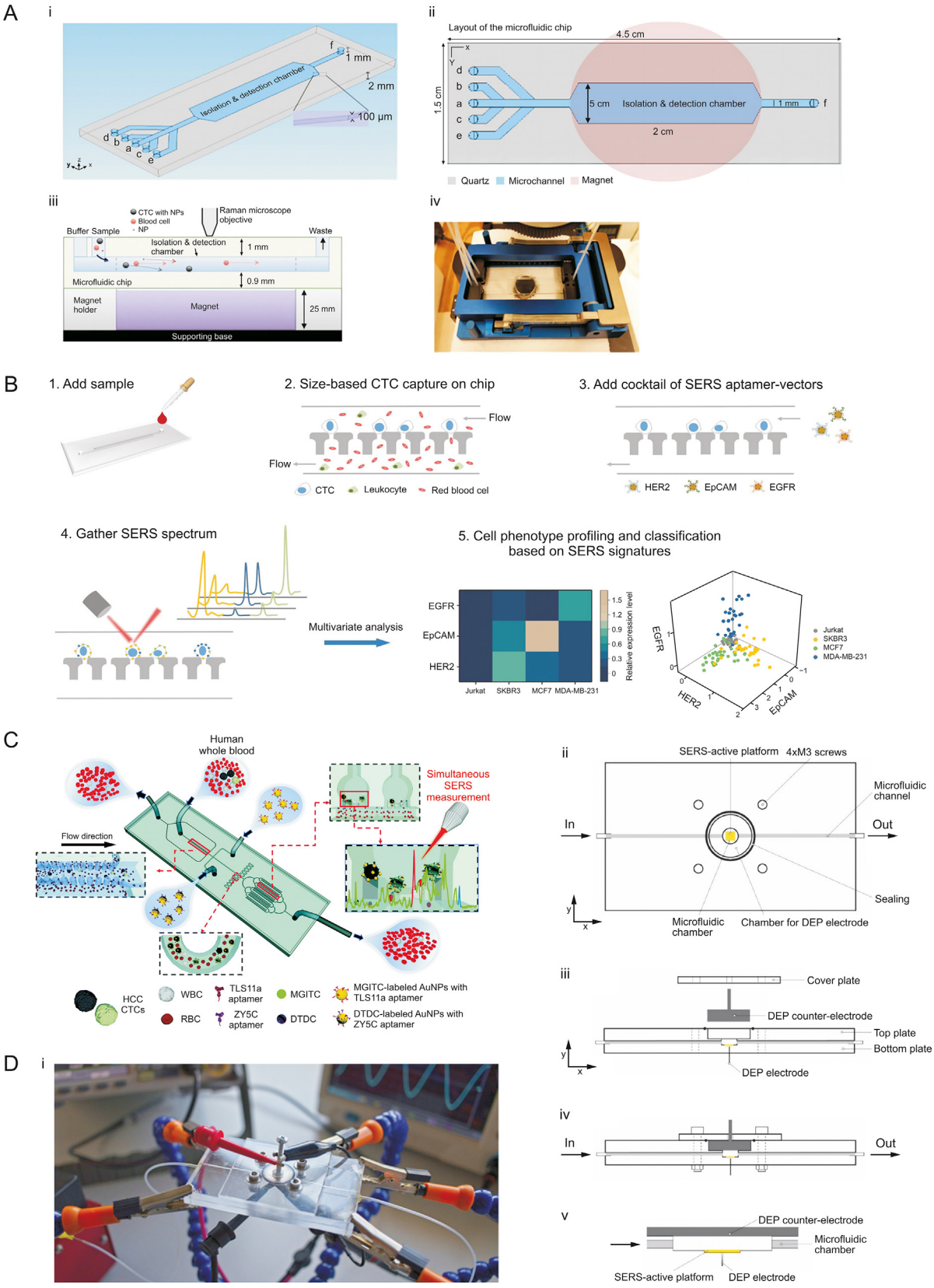


Fig. 5. Microfluidic chips based on surface-enhanced Raman scattering (lab-on-a-chip SERS (LoC-SERS)) for detection of circulating tumor cells (CTCs). (A) Design of the microfluidic device for on-chip magnetic isolation and optical detection of CTCs [203]. Three-dimensional (3D) view (i), layout (ii), and cross-sectional (iii) view of the microchannel, and actual picture of the microfluidic device (iv). Port (a) is used for supplying blood samples, (b) phosphate-buffered saline (PBS) for washing cells after each step, (c) paraformaldehyde solution for fixing cells, (d) fluorescein isothiocyanate-coupled CD45 antibody labeled leukocytes, (e) 4′,6-diamidino-2-phenylindole (DAPI) for restaining nucleus, and (f) is used as a waste outlet. (B) The workflow of operating on platform for size-based CTCs capture, profiling of cell phenotype, and classification based on SERS signatures [230]. (C) The workflow of SERS-aptamer-based microfluidic chip for hepatocellular carcinoma CTC capture, SERS measurement, and single-cell phenotype analysis [219]. (D) Views of the

laboratories. Nevertheless, they may cause some inconvenience to routine screening or POCT and have some shortcomings, such as complicated instrument operation, time-consuming exosome damage, and protein pollution [243–245]. For example, differential ultracentrifugation, the gold standard technique for isolating exosomes, requires cumbersome steps and expensive instrumentation, which may lead to low recoveries and high costs [246].

In addition to isolating exosomes, appropriate determination methods for exosomes are also necessary [247]. Some traditional techniques, such as protein blotting, ELISA, and MS, have limitations in exosome analysis due to low sensitivity, cumbersome procedures, and high sample handling requirements, which are not ideal for broader biological and medical research on exosomes [248]. Generally, separation, collection, and detection methods limit the efficiency of exosome analysis in terms of time and economic cost, operation friendliness, and detection accuracy, so new strategies are urgently needed to simplify the whole process. In recent years, the introduction of integrated microfluidic platforms has enabled the isolation and in situ molecular detection of exosomes, improving the efficiency of the analysis to a large extent [249,250]. The precise fluid control and high integration of various functions of microfluidic chips make them have a series of merits, including simple operation, easy automation, short analysis time, and less sample consumption [251]. The combination of microfluidic technology and various detection methods has been developing. Table 5 [252–259] lists several methods for exosome detection combined with microfluidic platforms. SERS can also be combined with the microfluidic platform for exosome analysis. In addition to high sensitivity and negligible water interference, the main advantage of SERS in exosome detection lies in its fingerprint characteristics and narrow spectral band, which makes it unique in multiple detections. As mentioned above, multiple detections of various target recognition substances (such as proteins on the surface of exosomes) are conducive to ensuring the accuracy of detection results.

5.3. Applications of LoC-SERS in exosome analysis

In the platform of LoC-SERS, most detection methods of exosomes are based on SERS labels, which are also considered to be more promising in clinical applications. In contrast, the complex background easily influences label-free identification in biological fluids [260]. The label-free detection of exosomes depends on the laser, sample, and SERS substrate, but it is not easy to coordinate the interaction of these three aspects [261]. Microfluidic chips are usually designed with the physical properties (such as the size of exosomes) and biological properties (such as immune affinity) of exosomes to maximize separation and detection. This section focuses on two representative LoC-SERS platforms based on SERS labels for exosome analysis.

5.3.1. SERS detection based on immunomagnetic separation

Magnetic enrichment can isolate and identify exosomes by integrating some exosome-specific surface proteins, such as EpCAM, CD63, CD9, ALIX, Annexin, and RAB5 [262]. Wang et al. proposed an LoC-SERS platform with magnetic separation for detecting prostate cancer, as shown in Fig. 6A [263]. The external sample and anti-CD63 magnetic beads are pumped at a certain speed through inlets 1 and 3, respectively. These two solutions are fully mixed and flow through the staggered triangular microcolumn mixing chamber. Under the action of the external magnetic field, the Mag-CD63-exo complex was immobilized in the Raman detection area. At this time, PBS buffer is introduced into inlet 2, and the mag-CD63-Exo complex in the mixing chamber is washed out. Next, EpCAM and functionalized Raman beads are introduced through inlet 4 and mixed with mag-CD63-Exo to form a sandwich immune complex. The exosomes were washed with PBS again, and finally, they were detected by Raman spectroscopy. The method can complete the detection within 1 h, and the LOD of a 20 μL sample is close to 160 exosomes/mL. The team used the LoC-SERS platform to determine 10 serum samples from patients diagnosed with PCa and 8 representatives from healthy individuals, which showed good discrimination ability. The advantage of using this platform to analyze exosomes is that all the operation steps, including mixing, separation, enrichment, and detection, are completed on the chip, significantly saving time and reflecting the microfluidic platform's integration and operation friendliness.

5.3.2. SERS detection based on size separation

According to the size difference of exosomes, combining a microfluidic chip and filter membrane can also realize the separation of exosomes. Han et al. designed a LoC-SERS platform to diagnose osteosarcoma, as shown in Fig. 6B [264]. In this study, SERS labels were prepared by functionalizing gold nanoparticles (AuNPs) with specific antibodies (CD63, vimentin, and EpCAM) and Raman reporters (4-MBA and 2,3,5,6-tetrafluoro-4-mercaptobenzoic acid (TFMBA)). SERS labels are mixed with the sample, incubated, and introduced into the microfluidic device with tangential flow filtration. Unbound SERS tags were filtered out due to their small size, while exosome immune complexes were enriched on the membrane surface for in situ SERS analysis. The LOD of this method reached 2 exosomes/μL, and only 50 μL of plasma was consumed in one analysis. On one hand, the study analyzed the multiple protein levels of exosomes by SERS, which helped improve the accuracy of exosome detection. On the other hand, the platform used tangential flow filtration, which can effectively avoid the blockage of filter membranes, thus improving the analysis efficiency.

6. LoC-SERS used to detect nucleic acid markers

6.1. ctDNA in liquid biopsy

Analysis of circulating DNA is an integral part of precision cancer treatment, especially blood-based ctDNA detection, which provides a minimally invasive and easy-to-realize method for cancer diagnosis, prognosis judgment, and treatment guidance [16]. In 1948, Mandel et al. [265] first discovered the existence of acellular nucleic acids in the blood of healthy and sick individuals. Leon et al. [266] found that the concentration of cell-free DNA (cfDNA) in cancer patients was much higher than that in healthy individuals in 1977 and expounded the possible application of circulating tumor DNA in oncology. Simply put, ctDNA is a subclass of cfDNA, approximately 130–150 base pairs, that falls off from the tumor and enters the circulation during cell apoptosis and necrosis [7,267–269]. ctDNA maintains the same genome changes in the corresponding tumors, thus allowing quantitative and qualitative

microfluidic chip for the dielectrophoretic deposition of CTCs on the surface of the SERS platform [231]. Actual picture (i), schematic diagram (ii), chip cross-section before (iii) and after (iv) chip assembly, and detailed view of the microfluidic chamber with dielectrophoresis (DEP) electrode under the SERS platform and counter-electrode at the top of the chamber (v). NPs: magnetic-optical nanoparticles; HER2: human epidermal growth factor receptor 2; EpCAM: epithelial cell adhesion molecule; EGFR: human epidermal growth factor receptor 1; HCC: hepatocellular carcinoma; WBC: white blood cell; RBC: red blood cell; MGITC: malachite green isothiocyanate; DTDC: 3,3′-diethylthiadicarbocyanine iodide. Reprinted from Refs. [203,219,230,231] with permission.

Table 5
Methods utilized for the detection of exosomes in combination with microfluidic platforms.

Related cancer types	Target	Isolation method	Detection method	Limit of detection	Refs.
Ovarian cancer	EpCAM	Immunomagnetic separation	Fluorescence	7.5×10^5 particles/mL	[252]
Lung cancer	PD-L1	Centrifugal microfluidic disc system	Aptamer fluorescence system	1.58×10^5 particles/mL	[253]
–	CD-63	Aptamer capture	Electrochemical	1×10^6 particles/mL	[254]
Breast cancer	EpCAM	Aptamer capture	Electrochemical	17 exosomes/μL	[255]
–	CD-63	Antibody capture	Surface plasmon resonance	1 ng/mL	[256]
Breast cancer	PD-L1	Antibody capture	Surface plasmon resonance	PD-L1 (high): 1.2×10^3 particles/μL PD-L1 (low): 6.3×10^3 particles/μL	[257]
–	CD-63	Antibody capture	Graphene field-effect transistor	0.1 μg/mL	[258]
–	CD-63	Antibody capture	Si nanowire field-effect transistor	2159 particles/mL	[259]

–: no data. EpCAM: epithelial cell adhesion molecule; PD-L1: programmed cell death ligand 1; CD-63: lysosomal membrane-associated glycoprotein 3.

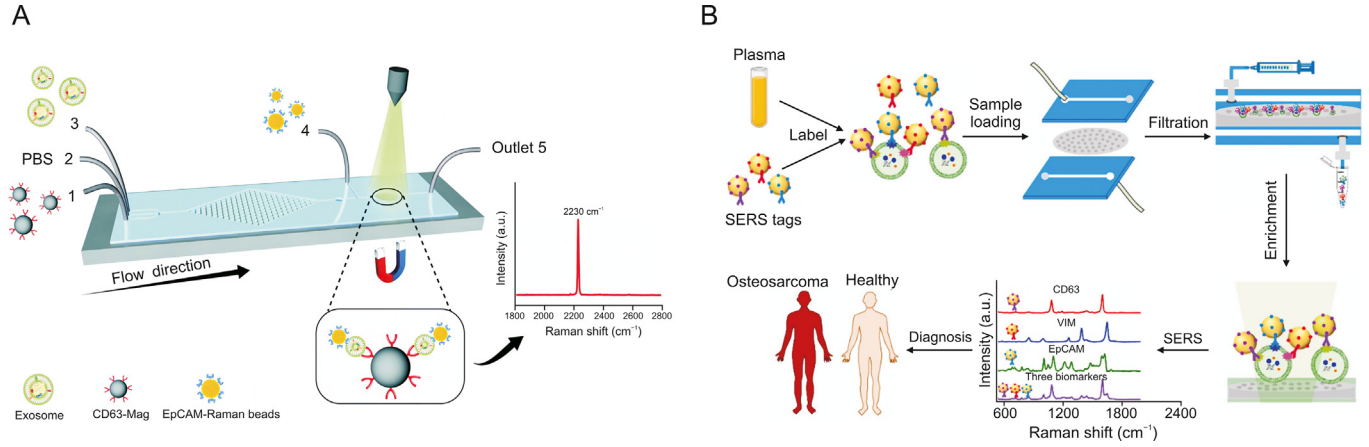


Fig. 6. Microfluidic chips based on surface-enhanced Raman scattering (lab-on-a-chip SERS (LoC-SERS)) for detection of exosomes. (A) Working principle of the microfluidic Raman chip based on continuous flow mixing and immunomagnetic isolation for exosome capture and detection [263]. (B) Schematic illustration of SERS profiling of three biomarkers on plasma-derived exosomes isolated by size-dependent microfluidic filtration to diagnose osteosarcoma [264]. PBS: phosphate-buffered saline; CD63-Mag: anti-CD63 magnetic nanoparticles; EpCAM: epithelial cell adhesion molecule; VIM: vimentin. Reprinted from Refs. [263,264] with permission.

real-time evaluation in body fluids [270], such as ctDNA mutation, copy number variation, and DNA methylation [271,272]. Although ctDNA has certain potential as a biomarker for cancer diagnosis, the concentration level of ctDNA in early patients is much lower than that in advanced patients [269,273], which is a challenge to the sensitivity of detection methods. It is worth noting that ctDNA has a very short half-life, mainly representative of the actual situation. It may reflect dynamic molecular changes during tumor development [274].

6.2. miRNAs in liquid biopsy

miRNAs have also become promising biomarkers in cancer diagnosis and monitoring in the past decade [275]. miRNAs were first discovered in 1993 and belong to a class of noncoding RNAs consisting of 19–25 nucleotides [276,277]. Circulating miRNAs are a typical subgroup, defined as extracellular miRNAs, and may be found in or outside blood vessels [278,279]. miRNA biogenesis starts from the nucleus and is then processed in the cytoplasm, which is a complicated process [280]. The diversity and quantity of miRNAs are usually related to the organism's complexity, but many miRNAs are highly conserved in the process of evolution [281]. The number of mature human miRNAs reported thus far exceeds 2600 [282]. It has been reported that approximately 60% of mRNA will be regulated by miRNA, which, in addition to its paradigm function of binding and inhibiting the translation of its target RNA, also includes translation upregulation, transcription activation, epigenetic regulation, and their existence in mitochondria and nuclei [282,283]. miRNAs participate in almost every major biological process, including proliferation, apoptosis, differentiation, organogenesis, etc., and are necessary for the normal development of animals [284,285]. At the same time, the imbalance of miRNAs is related to cancer [286,287]. It has been reported that the level of some specific miRNAs in the blood of cancer patients is higher than that of healthy individuals [280]. As intercellular signaling molecules mediating intercellular communication, miRNAs can be secreted into extracellular fluids, becoming a potential noninvasive biomarker [276]. To date, many studies on circulating miRNAs related to various cancers have been reported. For example, a variety of miRNAs, such as *miR-148a, miR-30c, miR-29b, miR-31, miR-30b-5p, miR-92a, miR-21, miR-5088-5p*, can be regarded as potential biomarkers in the study of female breast cancer [288–292].

6.3. Analysis methods of nucleic acid biomarkers

At present, the detection methods of ctDNA mainly include quantitative PCR (qPCR), digital drop PCR, and NGS, especially NGS, which significantly improves the detection sensitivity and specificity of ctDNA detection and characterization and marks the beginning of the “golden age” of liquid biopsy [273]. Similar to ctDNA detection, miRNA detection mainly includes qPCR or NGS, in addition to microarray analysis. Table 6 [22–25,293–301] shows several methods for detecting nucleic acid biomarkers. Although the above methods for detecting ctDNA and circulating ctmiRNA

Table 6
Methods utilized for the detection of circulating tumor DNAs (ctDNAs) and microRNA (miRNAs).

Related cancer type	Sample type	Category	Specific target	Detection method	Limit of detection	Refs.
Colorectal cancer	Tissue	miRNA	*let-7a*	Real-time quantitative PCR	10 copies/μL	[22]
Lung cancer	Tissue	miRNA	*miR-126*	Droplet digital PCR	10 copies/μL	[23]
Breast cancer	Tissue and blood	miRNA	–	Next generation sequencing	–	[24]
Oral squamous cell carcinoma	Tissue	miRNA	*miR-21-3p*	Next generation sequencing	–	[25]
Thyroid cancer	Blood	ctDNA	*BRAF V600E*	Electrochemical-enrichment assisted amplification refractory mutation system-quantitative real-time PCR	2 copies in Tris buffer 25 copies in 50% plasma	[293]
Lung cancer	Blood	ctDNA	*EGFR L858R*	Digital PCR	5 copies/μL	[294]
–	Blood	ctDNA	*EGFR T790 M*	Digital droplet PCR	1% mutant frequency	[295]
Lung cancer	Blood	ctDNA	*EGFR exon 21* and *EGFR L858R*	Digital droplet PCR	10 copies/μL	[296]
Ovarian cancer	Blood	miRNA	*miR-200b*	Graphene oxide-based qRT-PCR	10 copies/μL	[297]
Breast cancer	Blood	ctDNA	*TP53* and *PIK3CA*	Molecular barcode next-generation sequencing	0.1% variant allele frequency	[298]
Malignant melanoma	Blood	ctDNA	*BRAF V600*	Microarray and droplet digital PCR	0.05% mutated DNA from wild-type DNA background	[299]
Colorectal cancer	Tissue and blood	ctDNA	*KRAS*, *NRAS*, and *BRAF*	Tag-microarray method	0.03%–0.28% variant allele frequency	[300]
Colorectal cancer	Tissue	miRNA	–	Microarray analysis	–	[301]

–: no data; PCR: polymerase chain reaction; BRAF: v-raf murine sarcoma viral oncogene homolog B1; EGFR: epidermal growth factor receptor; qRT-PCR: quantitative real-time fluorescence PCR; TP53: tumor protein p53 gene; PIK3CA: phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; KRAS: Kirsten rat sarcoma viral oncogene homolog; NRAS: neuroblastoma RAS viral oncogene homolog.

are widely used, POCT must have lower sample consumption, faster turnaround time, and higher experimental yield, and many of the above methods are used for tissue detection. Nevertheless, they may not be suitable for body fluid detection [302].

6.4. Applications of LoC-SERS in nucleic acid biomarker analysis

Addressing the detection of ultrasensitive targets with low concentrations, such as ctDNA or miRNA, is an urgent matter that cannot be overlooked. The catalytic hairpin assembly (CHA) reaction, as a nonenzymatic and isothermal amplification strategy based on dynamic DNA self-assembly, has been proven to be able to amplify and transduce signals to detect various RNAs and DNAs, showing high sensitivity and selectivity [303,304]. As a general amplification strategy, CHA can be triggered by the target only by designing two hairpin DNAs (hpDNAs) and then self-assembling them into a double-stranded DNA structure through base pairing. The CHA strategy enables release and reuse from the target, making it an ideal method for constructing a multifunctional signal amplification strategy [305]. In addition, the loop structure of hpDNA can be opened by one-step hybridization with the target nucleic acid. The signal intensity change of Raman signal molecules linked to hpDNA can also be used to determine nucleic acids. Moreover, there are also studies to improve detection accuracy by combining various detection methods or designing microfluidic chips for the secondary detection of samples.

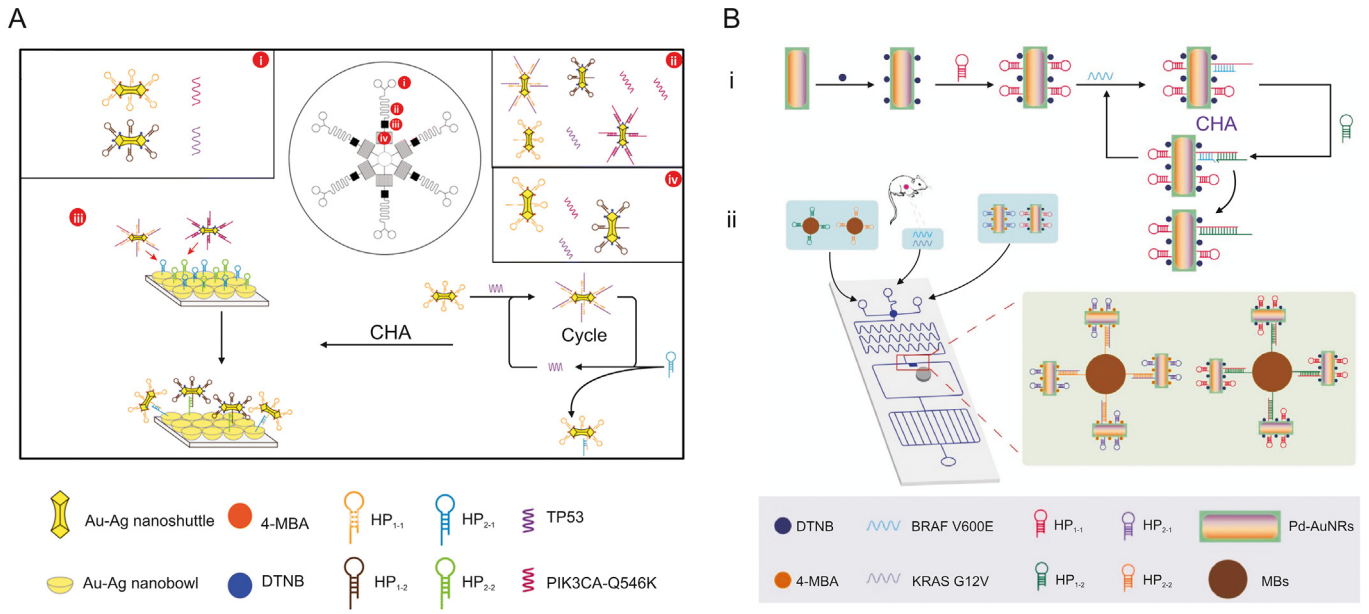
6.4.1. CHA strategy used in LoC-SERS

Combining the CHA strategy and SERS analysis further improves sensitivity and specificity. Meanwhile, the presence of microfluidic chips makes nucleic acid detection more compatible with the requirements of POCT. Cao et al. used this combined technique to detect two kinds of ctDNA (*TP53* and *PIK3CA-Q546K*) associated with non-small cell lung cancer. The microfluidic chip has six parallel detection channels, as shown in Fig. 7A [306]. Two kinds of Raman signal molecules (4-MBA and DTNB) and two hpDNAs (HP1-1 and HP1-2) were modified on the Au-Ag nanoshuttle surface to form two probes. The other two hpDNAs (HP2-1 and HP2-2) were modified on the surface of Au-Ag nanobowls in a microfluidic chip to form capture substrates. First, SERS probes and sample solution are dropped into the injection regions of the microfluidic chip (consisting of two 2-mm diameter circular holes), and the dripped solutions can flow spontaneously and fully contact each other under capillary force. Then, the CHA reaction occurred on the surface of highly ordered Au-Ag nanobowl arrays (EF = 7.02×10^8). With the progress of the reaction, an increasing number of SERS probes gather on the surface of the Au-Ag nanobowl array to produce many hot spots, thus enhancing the Raman signal. Through this method, the team realized the sensitive detection of *TP53* and *PIK3CA-Q546K* in human serum, and the LODs were as low as 2.26 and 2.34 aM, respectively.

Cao et al. also designed a microfluidic platform to simultaneously detect two other non-small cell lung cancer-related ctDNAs, *BRAF V600E* and *KRAS G12V*, as shown in Fig. 7B [307]. In the presence of a target, the CHA reaction can be triggered between two hpDNAs, immobilizing the Pd-Au core-shell nanorods on the surface of magnetic beads. Then, the composite structure can be assembled under magnetic force, realizing double amplification of SERS signals.

6.4.2. One-step paired hybridization reaction used in LoC-SERS

In addition to the above methods based on amplification technology, a one-step paired hybridization reaction is also suitable for detecting nucleic acid molecules. In short, in the reaction zone of a microfluidic chip, hpDNA labeled by Raman signal molecules is modified on the SERS substrate. Before detection, the signal molecules generate strong SERS signals near the substrate surface. In the event of miRNA target chains within the system, they will pair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B
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ii
CHA
DTNB
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4-MBA
KRAS G12V
HP1-2
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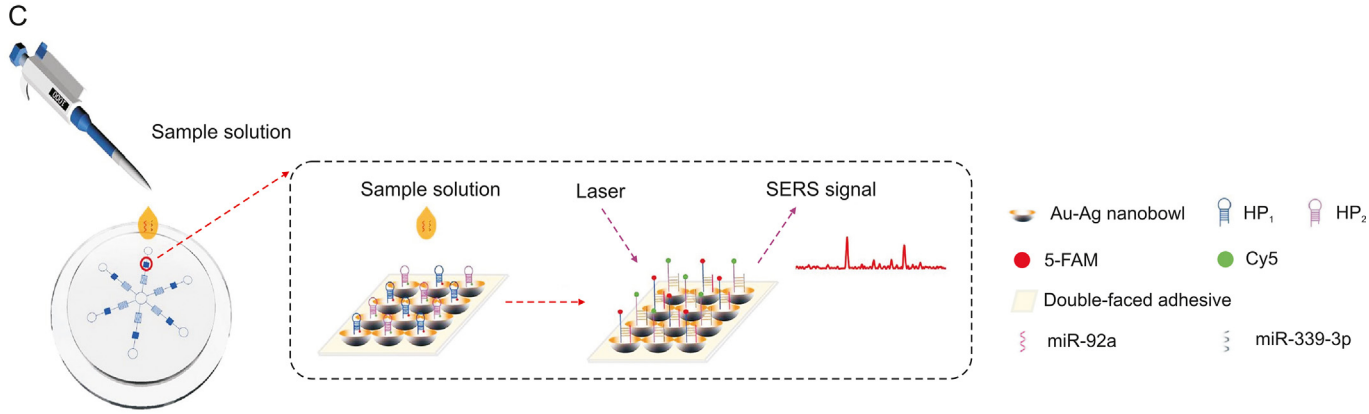
C
Sample solution
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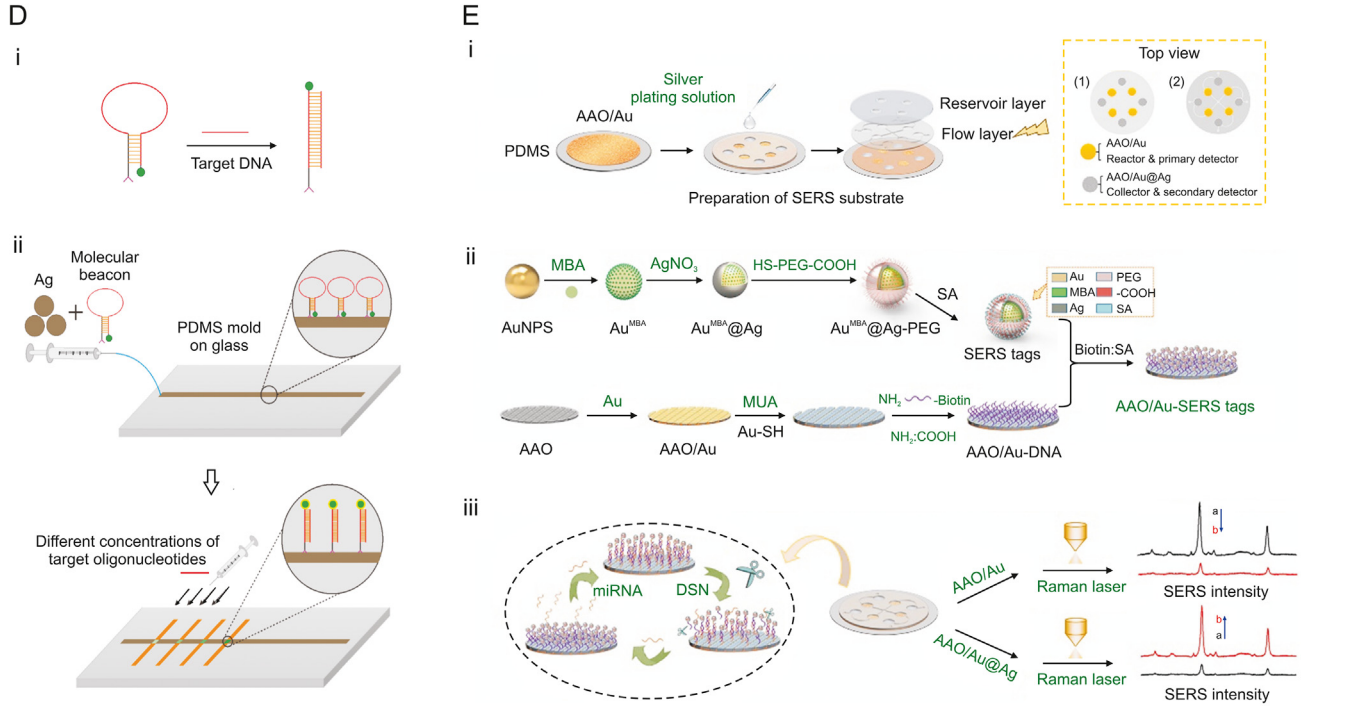
Laser
SERS signal
Au-Ag nanobowl
HP1
HP2
5-FAM
Cy5
Double-faced adhesive
miR-92a
miR-339-3p


D
i
Target DNA
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Molecular beacon
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PDMS mold on glass
Different concentrations of target oligonucleotides
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AAO/Au
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Reservoir layer
Flow layer
Preparation of SERS substrate
Top view
(1)
(2)
AAO/Au
Reactor & primary detector
AAO/Au@Ag
Collector & secondary detector
ii
MBA
AgNO3
HS-PEG-COOH
SA
AuNPS
AuMBA
AuMBA@Ag
AuMBA@Ag-PEG
SERS tags
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PEG
MBA
-COOH
Ag
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Biotin:SA
AAO/Au-SERS tags
Au
MUA
NH2 -Biotin
AAO
AAO/Au
Au-SH
NH2:COOH
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iii
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and hybridize with the corresponding hpDNA, ultimately leading to the dissociation of the stem region of hpDNA. The opening of the loop structure keeps the signal molecules away from the substrate surface, which leads to the weakening of the SERS signal. With an increase in miRNA concentration, the SERS signal will be more attenuated, which can be quantitatively detected according to the signal change. Gu et al. successfully detected two miRNAs (*miR-92a* and *miR-339-3p*) related to non-small cell lung cancer by using a Au-Ag nanobowl array as the SERS substrate (EF = 7.54×10^8), and the LOD was as low as 44.36 and 63.58 aM, respectively, as shown in Fig. 7C [308].

6.4.3. Secondary detection in LoC-SERS

When detecting and analyzing single-point nucleic acid molecules, it is crucial to be aware of the potential for false-negatives and false-positives due to their small quantity and complicated background. Some studies use secondary detection to improve the accuracy of detection. For example, through the one-step paired hybridization reaction mentioned above, Wang et al. successfully detected *miRNA-21* by combining SERS and fluorescence, as shown in Fig. 7D [309]. This study labeled the hairpin structure with the organic dye 6-carboxyfluorescein (6-FAM), which was used as a fluorophore and SERS reporter molecule. Due to the hairpin structure, when no target miRNA is present, 6-FAM becomes close to the nanosubstrate. This results in generating a strong Raman signal and simultaneous quenching of the fluorescence signal. When the target miRNA appears, it hybridizes with hpDNA molecules to open the hairpin structure, and the distance between 6-FAM and silver nanoparticles increases. Therefore, the fluorescence of 6-FAM will be restored, and the SERS signal of 6-FAM will be weakened. To some extent, this dual detection method can compensate for the false-positive or false-negative test results that sometimes arise from single-point detection.

The functional design of the microfluidic chip allows for the secondary detection of analytes, which can also effectively solve the problems above. Ma et al. designed a microfluidic device with dual detection zones. They constructed a SERS biosensor based on microfluidic technology using target cycle amplification technology assisted by duplex-specific nuclease (DSN), which also realized the sensitive detection of single-point *miRNA-21*, as shown in Fig. 7E [302]. This study divides the functionalized porous anodic aluminum oxide (AAO) substrate into a reaction chamber with an AAO/Au array and a secondary detection chamber with an AAO/Au@Ag array. SERS nanotags (Au^{MBA}@Ag core-shell nanoparticles) were immobilized on AAO/Au arrays by single-stranded DNA (ssDNA). When *miRNA-21* is close to the surface of the AAO/Au array, it hybridizes with ssDNA complementarily, forming a DNA/miRNA heteroduplex. Then, the target recovery process assisted by DSN is triggered, and the newly formed DNA/miRNA heteroduplex is cut into DNA fragments and single-stranded miRNA by the DSN enzyme. SERS labels also dissociate from the nanosurface, which leads to the weakening of the SERS signal. The released target miRNA will further hybridize with other DNA probes on the AAO/Au array, and the suspended SERS nanotags will be transferred to the second detection chamber with the AAO/Au@Ag array to generate SERS signals. In this complementary detection mode, the detection of *miR-21* is realized in the wide range of 10 fM−10 nM.

7. Challenges

For the analysis of biomarkers, it is usually necessary to consider many factors when choosing the appropriate detection method, such as whether the properties of the tested substance match the detection principle and whether the detection results obtained by the selected method are reliable. On these bases, attention should also be paid to the method's sensitivity, selectivity, convenience, and time cost. Thus, the development of interdisciplinary detection methods in medicine makes it more intelligent, integrated, and convenient. This review introduces several typical LoC-SERS platforms for detecting various cancer biomarkers. However, it is undeniable that they are still in their early developmental phase, which is probably why the literature has been relatively limited in recent years.

On one hand, while liquid biopsies have great potential for future diagnostic, prognostic, and therapeutic monitoring of cancer patients, only three liquid biopsy trials have been approved by the FDA, two for cfDNA (Corbus EGFR mutation test v2 and Epi proColon) and one for CTC-based practices (Cell Search). The low approval rate is due to the generally low reproducibility of preclinical studies [10]. Taking miRNA as an example, at the present level of knowledge, different cancer research groups still cannot obtain the same identification results of cell-free miRNA, so it is still a challenge to use a single cell-free miRNA for cancer diagnosis and prognosis in clinics [280]. To meet the technical requirements of the method, practice guidelines and standard operating procedures are indispensable, which are essential for generating reliable and reproducible data [310].

On the other hand, both microfluidic technology and SERS are relatively new analytical methods. Even if they are combined, there are still many challenges to be faced when they are put into clinical practice for routine detection and screening of cancer. First, one of the major advantages of SERS is its unique fingerprint identification, which makes it possible to detect nonmarkers. However, due to the complexity of liquid biopsy samples and the extremely low abundance of biomarkers in them, SERS signals are easily interfered with by the background. For example, when detecting exosomes in blood or serum, in addition to identifying a few exosomes from cancer cells from a large number of exosomes from different sources, it is necessary to overcome the complicated analysis signals caused by nonspecific adsorption of other molecules [260].

To solve the problem of Raman background interference, such as protein, lipid, and other endogenous substances and cell autofluorescence signal interference, the most common method is to purify samples before detection. For example, Phung et al. [311] used a borate extraction plate to extract the analyte before SERS

Fig. 7. Microfluidic chips based on surface-enhanced Raman scattering (lab-on-a-chip SERS (LoC-SERS)) for detection of nucleic acid biomarkers. (A) Principle of catalytic hairpin assembly (CHA) and SERS microfluidic chip for detecting circulating tumor DNA (ctDNAs) [306]. Injection region (i), mixing region (ii), reaction region (iii), and comb structure region (iv). (B) Schematic of ctDNA analysis based on the pump-free SERS microfluidic chip [307]. The preparation process for the SERS probes (i), SERS detection of *v-raf murine sarcoma viral oncogene homolog B1* (*BRAF*) *V600E* and *Kirsten rat sarcoma viral oncogene homolog* (*KRAS*) *G12V* based on the proposed platform (ii). (C) Flow chart of the application of the SERS microfluidic chip for detection of *miR-92a* and *miR-339-3p* [308]. (D) Microfluidic chip based on the combination of fluorescence and SERS for detecting microRNA (miRNA) [309]. The working mechanism of the designed molecular beacon (i) and schematic diagram of the microfluidic assay of the target oligonucleotides (ii). (E) Schematic illustration of microfluidic SERS biosensor based on anodic aluminum oxide (AAO)/Au array and AAO/Au@Ag array for detecting *miRNA-21* [302]. The preparation of microfluidic SERS biosensor (i), preparation and immobilization of SERS nanotags (Au^{MBA}@Ag core-shell nanoparticles) (ii), Raman measurement combined with duplex-specific nuclease (DSN)-assisted target recycling amplification strategy for detection of *miRNA-21* (iii). 4-MBA: 4-mercaptobenzoic acid; DTNB: 5,5′-dithiobis-(2-nitrobenzoic acid); HP: hairpin DNA; Pd-AuNRs: Pd-Au core-shell nanorods; MBs: magnetic beads; 5-FAM: 5-carboxyfluorescein; Cy5: cyanine 5; PDMS: polydimethylsiloxane; AuNPs: gold nanoparticles; HS-PEG-COOH: thiol (polyethylene glycol) acid; SA: streptavidin; MUA: 11-mercaptoundecanoic acid. Reprinted from Refs. [302,306–309] with permission.

detection of dopamine in plasma samples, and the background signal of the Raman spectrum was reduced. The simplicity of the method should be considered when the sample is pretreated; otherwise, it may be unfavorable to the implementation of POCT. There are studies on signal correction by adding internal standards, which can also avoid the background interference of complex samples [312,313]. In addition, some studies have used the Raman silent strategy [314–316]. By designing the Raman probe, it can generate Raman signals in a specific Raman shift range, that is, the Raman silent region (1800–2800 cm^{-1}), while most biomolecules do not generate Raman signals in this region, thus achieving the purpose of eliminating background interference.

Increasing the specific surface area of SERS substrate nanostructures makes it easier to obtain abundant hot spots, especially as nanotechnology is booming today. However, if their signals are uneven and unstable, it will be difficult to go further to the level of clinical diagnosis and application; therefore, many researchers are devoted to finding highly ordered SERS substrates to make hot spots evenly distributed. The microfluidic device mentioned in this paper can also improve the repeatability and stability of the SERS signal. However, at present, it is still necessary to standardize regulations such as SERS substrate preparation and performance control of microfluidic chips to provide reliable references.

It is also worth mentioning that the quantification of most biomarkers is based on SERS intensity, and the intensity signal often fluctuates inevitably due to the heterogeneity of the SERS substrate, thus affecting the reproducibility of detection. In 2012, Olivo and co-workers [317] proposed a quantitative immunoassay method based on the SERS frequency shift. They found that the vibration frequency of antibody-coupled SERS activity reporter molecules would change quantitatively with the change in target antigen concentration. In summary, this is mainly due to mechanical transduction, charge transfer, or local electric field effects [318]. In the next few years, studies on applying SERS frequency shift analysis in biomolecules have also been reported. Ma et al. [319] proposed a sandwich immunoassay that combined two independent SERS probes, 4-MBA and DSNB. Among them, the determination of alpha-fetoprotein (AFP) was realized through the evaluation of the 4-MBA frequency shift of the Raman signal molecule, and the logarithmic value of the AFP concentration had a good linear relation with the 4-MBA frequency shift value. This method is suitable for detecting macromolecular substances such as nucleic acids and proteins. Nevertheless, not all Raman reporter molecules will produce a Raman signal frequency shift after binding with the analyte. Thus, the development of reporter molecules and probes is also an issue that needs to be faced if this strategy is to be further expanded.

For microfluidic devices, miniaturization, portability, and integration are the basic requirements. First, regarding POCT, avoiding the use of complex and huge external equipment is necessary. For example, most microfluidic platforms mentioned in this paper use capillary action to complete the automatic driving of fluid on microfluidic platforms. In addition, the cost and capacity should also be considered to facilitate the mass production of microfluidic platforms. Most microfluidic platforms are made of cheap glass, polymers (such as PDMS), etc., and some studies have made paper the assembly material of microfluidic platforms to further reduce the cost. At the same time, to simplify production, combining other technologies, such as 3D printing, to develop a microfluidic platform can achieve the advantages of low cost, less waste, and easy design. Although this technology has shortcomings, such as limited resolution, it can still be used as a supplementary technology for microfluidic production [320].

Finally, the design of microfluidic platforms should provide convenient conditions according to the characteristics of SERS detection, such as sample pretreatment steps such as separation, mixing, and enrichment, through the design of microfluidic channels so that analytes can present the best form for SERS analysis. In addition, introducing the SERS substrate into the microfluidic channels is also the key to preparing the platform, which relates to the uniformity of the substrate in the channels and the uniformity of the detection signal. Traditional methods, such as injecting colloidal nanoparticles into the microfluidic channel, then adding the analyte solution, mixing, and analyzing, are challenging to achieve a consistent detection signal. In recent years, there have been continuous methods to synthesize SERS substrates on microfluidic chips in situ, which is helpful to improve the above problems. For example, Dugandžić et al. [28] obtained silver nanoparticles in glass microfluidic chips based on segmented flow by reducing silver ions with hydrazine in ammonium hydroxide solution, which showed long-term reproducibility, lower LOD, and wider working range in the detection of adenine. Parisi et al. [321] prepared silver nanoparticle-based active SERS substrates by electroplating replacement of prepatterned Cu in microfluidic channels and detected crystal violet and pesticides. The shape and size of the SERS detection area were controlled by prepatterned Cu substrates, allowing precise control of position, high reproducibility, and consistent results.

In summary, the high standards required for detection methods in cancer diagnosis present significant opportunities for further development and refinement of both microfluidic technology and SERS detection methods. While their combination holds great promise in precisely detecting cancer, there is still much work to be done to fully realize their clinical potential.

8. Conclusion

This review introduces several common biomarkers in cancer liquid biopsy, including circulating proteins, CTCs, exosomes, miRNA, and ctDNA. For the past few years, the continued discovery and study of these predictive biomarkers have led to the enormous potential of liquid biopsies in cancer diagnosis. The combination of microfluidic platforms and SERS is highly compatible and advantageous in biomarker detection research. With microfluidic devices providing precise control over liquids, the mixing, concentration, capture, and other steps of biomarkers can be completed centrally on the chip. SERS then quickly, sensitively, and accurately identifies and analyzes biomarkers. Both of them have POCT potential, which makes the liquid biopsy process more efficient and convenient. However, it is necessary to note that the full potential of this technique may not yet be fully realized. For example, the label-free SERS assay should have performed a much more powerful function in screening biomolecules. Nevertheless, due to the complexity of biological samples, label-based SERS assays are mostly in use. In addition, the reproducibility and stability of SERS signals, the standardization of platform production, and the cost and capacity of pushing the platform into production should be considered. Therefore, there are still a series of challenges if this combined technique is applied to the clinical detection of cancer biomarkers. However, we believe that with an increasing number of people paying attention to and participating in research in this field, these problems will be gradually overcome, and this potential combined technique will have a bright future in liquid biopsy.

CRediT author statement

Changhong Nie: Methodology, Investigation, Writing - Original draft preparation, Reviewing and Editing, Visualization, Software, Data curation; **Ibrahim Shaw:** Writing - Reviewing and Editing; **Chuanpin Chen:** Conceptualization, Supervision, Writing - Reviewing and Editing, Validation.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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References

[1] H. Sung, J. Ferlay, R.L. Siegel, et al., Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA 71 (2021) 209–249.
[2] R. Haldavnekar, K. Venkatakrishnan, B. Tan, Cancer stem cell derived extracellular vesicles with self-functionalized 3D nanosensor for real-time cancer diagnosis: Eliminating the roadblocks in liquid biopsy, ACS Nano 16 (2022) 12226–12243.
[3] M. Constantinou, K. Hadjigeorgiou, S. Abalde-Cela, et al., Label-free sensing with metal nanostructure-based surface-enhanced Raman spectroscopy for cancer diagnosis, ACS Appl. Nano Mater. 5 (2022) 12276–12299.
[4] F. Ferrara, S. Zoupanou, E. Primiceri, et al., Beyond liquid biopsy: Toward non-invasive assays for distanced cancer diagnostics in pandemics, Biosens. Bioelectron. 196 (2022), 113698.
[5] M. He, Y. Zeng, Microfluidic exosome analysis toward liquid biopsy for cancer, J. Lab. Autom. 21 (2016) 599–608.
[6] G. De Rubis, S. Rajeev Krishnan, M. Bebawy, Liquid biopsies in cancer diagnosis, monitoring, and prognosis, Trends Pharmacol. Sci. 40 (2019) 172–186.
[7] L. De Mattos-Arruda, G. Siravegna, How to use liquid biopsies to treat patients with cancer, ESMO Open 6 (2021), 100060.
[8] M. Jiang, J. Zhou, X. Xie, et al., Single nanoparticle counting-based liquid biopsy for cancer diagnosis, Anal. Chem. 94 (2022) 15433–15439.
[9] L. Wu, Y. Wang, L. Zhu, et al., Aptamer-based liquid biopsy, ACS Appl. Bio Mater. 3 (2020) 2743–2764.
[10] E. Geeurickx, A. Hendrix, Targets, pitfalls and reference materials for liquid biopsy tests in cancer diagnostics, Mol. Aspect. Med. 72 (2020), 100828.
[11] M. Zhao, D. Mi, B.E. Ferdows, et al., State-of-the-art nanotechnologies for the detection, recovery, analysis and elimination of liquid biopsy components in cancer, Nano Today 42 (2022), 101361.
[12] Z. Tang, J. Huang, H. He, et al., Contributing to liquid biopsy: Optical and electrochemical methods in cancer biomarker analysis, Coord. Chem. Rev. 415 (2020), 213317.
[13] K.K. Jain, Biomarkers of cancer. The Handbook of Biomarkers, Humana Press, New York, 2017, pp. 273–462.
[14] S. Bratulic, F. Gatto, J. Nielsen, The translational status of cancer liquid biopsies, Regen. Eng. Trans. Med. 7 (2021) 312–352.
[15] S.H. Hussain, C.S. Huertas, A. Mitchell, et al., Biosensors for circulating tumor cells (CTCs)-biomarker detection in lung and prostate cancer: Trends and prospects, Biosens. Bioelectron. 197 (2022), 113770.
[16] X. Han, J. Wang, Y. Sun, Circulating tumor DNA as biomarkers for cancer detection, Genomics Proteomics Bioinformatics 15 (2017) 59–72.
[17] H.M. Heneghan, N. Miller, M.J. Kerin, MiRNAs as biomarkers and therapeutic targets in cancer, Curr. Opin. Pharmacol. 10 (2010) 543–550.
[18] N.A. Hanjani, N. Esmaelizad, S. Zanganeh, et al., Emerging role of exosomes as biomarkers in cancer treatment and diagnosis, Crit. Rev. Oncol. Hematol. 169 (2022), 103565.
[19] R. Aebersold, L. Anderson, R. Caprioli, et al., Perspective: A program to improve protein biomarker discovery for cancer, J. Proteome Res. 4 (2005) 1104–1109.
[20] A. Plumer, H. Duan, S. Subramaniam, et al., Development of fragment-specific osteopontin antibodies and ELISA for quantification in human metastatic breast cancer, BMC Cancer 8 (2008), 38.
[21] M. Lim, P. Erdman, S. Cho, et al., Evaluation of CisBio ELISA for chromogranin A measurement, J. Appl. Lab. Med. 4 (2019) 11–18.
[22] W.J. Fang, C.Z. Lin, H.H. Zhang, et al., Detection of *let-7a* microRNA by real-time PCR in colorectal cancer: A single-centre experience from China, J. Int. Med. Res. 35 (2007) 716–723.
[23] P. Wang, F. Jing, G. Li, et al., Absolute quantification of lung cancer related microRNA by droplet digital PCR, Biosens. Bioelectron. 74 (2015) 836–842.
[24] Q. Wu, Z. Lu, H. Li, et al., Next-generation sequencing of microRNAs for breast cancer detection, J. Biomed. Biotechnol. 2011 (2011), 597145.
[25] H.-H. Tseng, Y.-K. Tseng, J.-J. You, et al., Next-generation sequencing for microRNA profiling: *microRNA-21-3p* promotes oral cancer metastasis, Anticancer Res. 37 (2017) 1059–1066.
[26] Y. Li, Z. Huang, Z. Li, et al., Mass spectrometric multiplex detection of microRNA and protein biomarkers for liver cancer, Anal. Chem. 94 (2022) 17248–17254.
[27] Y. Zhu, Y. Lian, J. Wang, et al., Ultrasensitive detection of protein biomarkers by MALDI-TOF mass spectrometry based on $ZnFe_2O_4$ nanoparticles and mass tagging signal amplification, Talanta 224 (2021), 121848.
[28] V. Dugandžić, I.J. Hidi, K. Weber, et al., *In situ* hydrazine reduced silver colloid synthesis - enhancing SERS reproducibility, Anal. Chim. Acta 946 (2016) 73–79.
[29] X. Cao, S. Ge, M. Chen, et al., LoC-SERS platform integrated with the signal amplification strategy toward Parkinson's disease diagnosis, ACS Appl. Mater. Interfaces 15 (2023) 21830–21842.
[30] A. März, P. Rösch, T. Henkel, et al., Lab-on-a-chip surface-enhanced Raman spectroscopy. Optical Nano- and Microsystems for Bioanalytics, Springer, Heidelberg, 2012, pp. 229–245.
[31] J. Sun, Y. Xianyu, X. Jiang, Point-of-care biochemical assays using gold nanoparticle-implemented microfluidics, Chem. Soc. Rev. 43 (2014) 6239–6253.
[32] A. Manz, N. Graber, H.M. Widmer, Miniaturized total chemical analysis systems: A novel concept for chemical sensing, Sens. Actuators B Chem. 1 (1990) 244–248.
[33] J. Zhuang, J. Yin, S. Lv, et al., Advanced "lab-on-a-chip" to detect viruses – Current challenges and future perspectives, Biosens. Bioelectron. 163 (2020), 112291.
[34] N. Venugopal Menon, S.B. Lim, C.T. Lim, Microfluidics for personalized drug screening of cancer, Curr. Opin. Pharmacol. 48 (2019) 155–161.
[35] L.-Y. Hung, H.-W. Wu, K. Hsieh, et al., Microfluidic platforms for discovery and detection of molecular biomarkers, Microfluid. Nanofluidics 16 (2014) 941–963.
[36] N. Bargahi, S. Ghasemali, S. Jahandar-Lashaki, et al., Recent advances for cancer detection and treatment by microfluidic technology, review and update, Biol. Proced. Online 24 (2022), 5.
[37] S.T. Sanjay, G. Fu, M. Dou, et al., Biomarker detection for disease diagnosis using cost-effective microfluidic platforms, Analyst 140 (2015) 7062–7081.
[38] A.C.Q. Silva, C. Vilela, H.A. Santos, et al., Recent trends on the development of systems for cancer diagnosis and treatment by microfluidic technology, Appl. Mater. Today 18 (2020), 100450.
[39] M. Doherty, T. Wang, D.A. Lamprou, et al., Microfluidic technologies in tumour metabolism, Int. J. Pharm. 629 (2022), 122370.
[40] J.R. Lake, K.C. Heyde, W.C. Ruder, Low-cost feedback-controlled syringe pressure pumps for microfluidics applications, PLoS One 12 (2017), e0175089.
[41] X. Zhang, K. Xia, A. Ji, A portable plug-and-play syringe pump using passive valves for microfluidic applications, Sens Actuators B Chem. 304 (2020), 127331.
[42] S.M. Ha, W. Cho, Y. Ahn, Disposable thermo-pneumatic micropump for bio lab-on-a-chip application, Microelectron. Eng. 86 (2009) 1337–1339.
[43] C.H. Liu, G.B. Lee, A micropump using amplified deformation of resilient membranes through oil hydraulics, Microfluid. Nanofluidics 17 (2014) 393–400.
[44] A.R. Ameri, A. Imanparast, M. Passandideh-Fard, et al., A whole-thermoplastic microfluidic chip with integrated on-chip micropump, bioreactor and oxygenator for cell culture applications, Anal. Chim. Acta 1221 (2022), 340093.
[45] T. Ma, S. Sun, B. Li, et al., Piezoelectric peristaltic micropump integrated on a microfluidic chip, Sens. Actuators A Phys. 292 (2019) 90–96.
[46] H.H. Wang, T.J. Wu, S.J. Lin, et al., Dual light-activated microfluidic pumps based on an optopiezoelectric composite, J. Micromech. Microeng. 27 (2017), 125003.
[47] C. Szydzik, R.J. Brazilek, F. Akbaridoust, et al., Active micropump-mixer for rapid antiplatelet drug screening in whole blood, Anal. Chem. 91 (2019) 10830–10839.
[48] G. Cai, Y. Huang, B. Chen, et al., Modular design of centrifugal microfluidic system and its application in nucleic acid screening, Talanta 259 (2023), 124486.
[49] J.C. Yeo, Z. Wang, C.T. Lim, Microfluidic size separation of cells and particles using a swinging bucket centrifuge, Biomicrofluidics 9 (2015), 54114.
[50] S. Uhlig, M. Gaudet, S. Langa, et al., Electrostatically driven in-plane silicon micropump for modular configuration, Micromachines 9 (2018), 190.
[51] L. Yang, T. Ye, X. Zhao, et al., Design and fabrication of a microfluidic chip for particle size-exclusion and enrichment, Micromachines 12 (2021), 1218.
[52] B. Liu, M. Li, B. Tian, et al., A positive pressure-driven PDMS pump for fluid handling in microfluidic chips, Microfluid. Nanofluidics 22 (2018), 94.
[53] X. Wang, D. Zhao, D.T.T. Phan, et al., A hydrostatic pressure-driven passive micropump enhanced with siphon-based autofill function, Lab Chip 18 (2018) 2167–2177.
[54] H. Li, J.V. Sørensen, K.V. Gothelf, Quantitative detection of digoxin in plasma using small-molecule immunoassay in a recyclable gravity-driven microfluidic chip, Adv. Sci. 6 (2019), 1802051.
[55] X. Xiang, Q. Ye, Y. Shang, et al., Quantitative detection of aflatoxin B_1 using quantum dots-based immunoassay in a recyclable gravity-driven microfluidic chip, Biosens. Bioelectron. 190 (2021), 113394.
[56] S. Mohammadi, L.S. Busa, M. Maeki, et al., Novel concept of washing for microfluidic paper-based analytical devices based on capillary force of paper substrates, Anal. Bioanal. Chem. 408 (2016) 7559–7563.
[57] C. Sun, H. You, N. Gao, et al., Design and fabrication of a microfluidic chip to detect tumor markers, RSC Adv. 10 (2020) 39779–39785.
[58] G.J. Amador, Z. Ren, A.F. Tabak, et al., Temperature gradients drive bulk flow within microchannel lined by fluid-fluid interfaces, Small 15 (2019), e1900472.
[59] T.E. de Groot, K.S. Veserat, E. Berthier, et al., Surface-tension driven open microfluidic platform for hanging droplet culture, Lab Chip 16 (2016) 334–344.

[60] Q. Song, J. Sun, Y. Mu, et al., A new method for polydimethylsiloxane (PDMS) microfluidic chips to maintain vacuum-driven power using Parylene C, Sens Actuators B Chem. 256 (2018) 1122–1130.
[61] C.-H. Lee, C.-C. Hong, A disposable emulsion droplet generation lab chips driven by vacuum module for manipulation of blood cells. 2015 37th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC), August 25–29, 2015, Milan, Italy, 2015, pp. 8010–8013.
[62] C. Chuang, Y. Chiang, Bio-O-Pump: A novel portable microfluidic device driven by osmotic pressure, Sens Actuators B Chem. 284 (2019) 736–743.
[63] Z. Xu, C. Yang, C. Liu, et al., An osmotic micro-pump integrated on a microfluidic chip for perfusion cell culture, Talanta 80 (2010) 1088–1093.
[64] L. Ying, Q. Wang, Microfluidic chip-based technologies: Emerging platforms for cancer diagnosis, BMC Biotechnol. 13 (2013), 76.
[65] J. Žvirblytė, L. Mažutis, Microfluidics for cancer biomarker discovery, research, and clinical application. Microfluidics and Biosensors in Cancer Research, Springer, 2022, pp. 499–524.
[66] X. Fang, Microfluidic chip. Clinical Molecular Diagnostics, Springer, Singapore, 2021, pp. 357–375.
[67] C. Li, Special topic: Point-of-care testing (POCT) and *in vitro* diagnostics (IVDs), J. Anal. Test. 3 (2019) 1–2.
[68] E. Sarraf, D.M. Mathews, M.H. Tsai, et al., Optimizing the use of point of care testing devices for screening patients, J. Clin. Monit. Comput. 34 (2020) 411–419.
[69] L. Bueno, W.R. de Araujo, T.R.L.C. Paixão, Point of care (POC) medical biosensors for cancer detection. Medical Biosensors for Point Of Care (POC) Applications, Elsevier, Amsterdam, 2017, pp. 183–201.
[70] W. Jung, J. Han, J.W. Choi, et al., Point-of-care testing (POCT) diagnostic systems using microfluidic lab-on-a-chip technologies, Microelectron. Eng. 132 (2015) 46–57.
[71] J.V. Pagaduan, V. Sahore, A.T. Woolley, Applications of microfluidics and microchip electrophoresis for potential clinical biomarker analysis, Anal. Bioanal. Chem. 407 (2015) 6911–6922.
[72] Z.A. Duca, N.C. Speller, M.E. Cato, et al., A miniaturized, low-cost lens tube based laser-induced fluorescence detection system for automated microfluidic analysis of primary amines, Talanta 241 (2022), 123227.
[73] M. Hao, C. Li, R. Liu, et al., Detection of glutathione within single erythrocyte of different ages and pathological state using microfluidic chips coupled with laser induced fluorescence, Spectrochim. Acta A Mol. Biomol. Spectrosc. 149 (2015) 600–606.
[74] H. Bi, A.C. Fernandes, S. Cardoso, et al., Interference-blind microfluidic sensor for ascorbic acid determination by UV/vis spectroscopy, Sens Actuators B Chem. 224 (2016) 668–675.
[75] X. Pang, L.J. Carpenter, A.C. Lewis, Microfluidic derivatisation technique for determination of gaseous molecular iodine with GC-MS, Talanta 137 (2015) 214–219.
[76] P. Chen, W. Zhang, W. Chen, et al., Engineering an integrated system with a high pressure polymeric microfluidic chip coupled to liquid chromatography-mass spectrometry (LC-MS) for the analysis of abused drugs, Sens Actuators B Chem. 350 (2022), 130888.
[77] L.J.K. Weiß, G. Lubins, E. Music, et al., Single-impact electrochemistry in paper-based microfluidics, ACS Sens. 7 (2022) 884–892.
[78] T. Sierra, I. Jang, E. Noviana, et al., Pump-free microfluidic device for the electrochemical detection of α_1-acid glycoprotein, ACS Sens. 6 (2021) 2998–3005.
[79] C.V. Raman, K.S. Krishnan, A new type of secondary radiation, Nature 121 (1928) 501–502.
[80] A. Shiohara, Y. Wang, L.M. Liz-Marzán, Recent approaches toward creation of hot spots for SERS detection, J. Photochem. Photobiol. C 21 (2014) 2–25.
[81] L. Long, W. Ju, H.-Y. Yang, et al., Dimensional design for surface-enhanced Raman spectroscopy, ACS Mater. Au 2 (2022) 552–575.
[82] A. Tycova, J. Prikryl, F. Foret, Recent strategies toward microfluidic-based surface-enhanced Raman spectroscopy, Electrophoresis 38 (2017) 1977–1987.
[83] Y. Jiang, D.-W. Sun, H. Pu, et al., Surface enhanced Raman spectroscopy (SERS): A novel reliable technique for rapid detection of common harmful chemical residues, Trends Food Sci. Technol. 75 (2018) 10–22.
[84] M.M. Joseph, N. Narayanan, J.B. Nair, et al., Exploring the margins of SERS in practical domain: An emerging diagnostic modality for modern biomedical applications, Biomaterials 181 (2018) 140–181.
[85] M. Fleischmann, P.J. Hendra, A.J. McQuillan, Raman spectra of pyridine adsorbed at a silver electrode, Chem. Phys. Lett. 26 (1974) 163–166.
[86] Y. Yuan, N. Panwar, S.H.K. Yap, et al., SERS-based ultrasensitive sensing platform: An insight into design and practical applications, Coord. Chem. Rev. 337 (2017) 1–33.
[87] Y. Qiu, C. Kuang, X. Liu, et al., Single-molecule surface-enhanced Raman spectroscopy, Sensors (Basel) 22 (2022), 4889.
[88] N.E. Dina, M.A. Tahir, S.Z. Bajwa, et al., SERS-based antibiotic susceptibility testing: Towards point-of-care clinical diagnosis, Biosens. Bioelectron. 219 (2023), 114843.
[89] B. Sharma, R.R. Frontiera, A.I. Henry, et al., SERS: Materials, applications, and the future, Mater. Today 15 (2012) 16–25.
[90] X. Wang, Y. Wu, X. Wen, et al., Composite structure of Au film/PMMA grating coated with Au nanocubes for SERS substrate, Opt. Mater. 121 (2021), 111536.
[91] R. Goodacre, D. Graham, K. Faulds, Recent developments in quantitative SERS: Moving towards absolute quantification, Trac Trends Anal. Chem. 102 (2018) 359–368.
[92] M. Li, Y. Qiu, C. Fan, et al., Design of SERS nanoprobes for Raman imaging: Materials, critical factors and architectures, Acta Pharm. Sin. B 8 (2018) 381–389.
[93] B. Shan, Y. Pu, Y. Chen, et al., Novel SERS labels: Rational design, functional integration and biomedical applications, Coord. Chem. Rev. 371 (2018) 11–37.
[94] H. Tang, C. Zhu, G. Meng, et al., Review – Surface-enhanced Raman scattering sensors for food safety and environmental monitoring, J. Electrochem. Soc. 165 (2018) B3098–B3118.
[95] A. Jabłońska, A. Jaworska, M. Kasztelan, et al., Graphene and graphene oxide applications for SERS sensing and imaging, Curr. Med. Chem. 26 (2019) 6878–6895.
[96] L. Wang, Y. Zhang, Y. Yang, et al., Strong dependence of surface enhanced Raman scattering on structure of graphene oxide film, Materials 11 (2018), 1199.
[97] G. Kim, M. Kim, C. Hyun, et al., Hexagonal boron nitride/Au substrate for manipulating surface plasmon and enhancing capability of surface-enhanced Raman spectroscopy, ACS Nano 10 (2016) 11156–11162.
[98] K. Ge, Q. Wu, Y. Li, et al., High and stable surface-enhanced Raman spectroscopy activity of h-BN nanosheet/Au_1Ag_3 nanoalloy hybrid membrane for melamine determination, Spectrochim. Acta A Mol. Biomol. Spectrosc. 271 (2022), 120952.
[99] Y. Liu, J. Zou, S. Chen, et al., Raman spectroscopy studies of black phosphorus, Spectrochim. Acta A Mol. Biomol. Spectrosc. 271 (2022), 120861.
[100] C. Lin, S. Liang, Y. Peng, et al., Visualized SERS imaging of single molecule by Ag/black phosphorus nanosheets, Nanomicro Lett. 14 (2022), 75.
[101] Z. Wu, D.-W. Sun, H. Pu, et al., Ti_3C_2Tx MXenes loaded with Au nanoparticle dimers as a surface-enhanced Raman scattering aptasensor for AFB1 detection, Food Chem. 372 (2022), 131293.
[102] Y. Peng, C. Lin, L. Long, et al., Charge-transfer resonance and electromagnetic enhancement synergistically enabling MXenes with excellent SERS sensitivity for SARS-CoV-2 S protein detection, Nanomicro Lett. 13 (2021), 52.
[103] H. Wu, X. Sun, C. Hou, et al., Preparation of quasi-three-dimensional porous Ag and Ag-NiO nanofibrous mats for SERS application, Sensors 18 (2018), 2862.
[104] Q. Zhou, G. Meng, Q. Huang, et al., Ag-nanoparticles-decorated NiO-nanoflakes grafted Ni-nanorod arrays stuck out of porous AAO as effective SERS substrates, Phys. Chem. Chem. Phys. 16 (2014) 3686–3692.
[105] L. Yang, X. Qin, X. Jiang, et al., SERS investigation of ciprofloxacin drug molecules on TiO_2 nanoparticles, Phys. Chem. Chem. Phys. 17 (2015) 17809–17815.
[106] X. Fu, G. Zhang, T. Wu, et al., Multifunctional gold-loaded TiO_2 thin film: Photocatalyst and recyclable SERS substrate, Can. J. Chem. 91 (2013) 1112–1116.
[107] H. Zhang, S. Huang, X. Yang, et al., A SERS biosensor constructed by calcined ZnO substrate with high-efficiency charge transfer for sensitive detection of Pb^{2+}, Sens Actuators B Chem. 343 (2021), 130142.
[108] X. Wang, W. Shi, Z. Jin, et al., Remarkable SERS activity observed from amorphous ZnO nanocages, Angew. Chem. Ed. Engl. 56 (2017) 9851–9855.
[109] I. Lettrichova, A. Laurencikova, D. Pudis, et al., 2D periodic structures patterned on 3D surfaces by interference lithography for SERS, Appl. Surf. Sci. 461 (2018) 171–174.
[110] A.M. Polubotko, V.P. Chelibanov, Group-theoretical interpretation of surface-enhanced Raman scattering spectra of copper phthalocyanine adsorbed on *Gallium* phosphide, Opt. Spectrosc. 126 (2019) 181–183.
[111] F. Yu, H. Huang, J. Shi, et al., A new gold nanoflower Sol SERS method for trace iodine ion based on catalytic amplification, Spectrochim. Acta A Mol. Biomol. Spectrosc. 255 (2021), 119738.
[112] Y. Luo, G. Wen, L. Ma, et al., A sensitive SERS quantitative analysis method for amino acids using Ruhemann's purple as molecular probe in triangle nanosilver sol substrate, Plasmonics 12 (2017) 299–308.
[113] G. Shang, C. Li, G. Wen, et al., A new silver nanochain SERS analytical platform to detect trace hexametaphosphate with a rhodamine S molecular probe, Luminescence 31 (2016) 640–648.
[114] X. Liang, G. Wen, Q. Liu, et al., Hydride generation-resonance Rayleigh scattering and SERS spectral determination of trace Bi, Spectrochim. Acta A Mol. Biomol. Spectrosc. 166 (2016) 95–102.
[115] Y. Luo, Q. Jing, C. Li, et al., Simple and sensitive SERS quantitative analysis of sorbic acid in highly active gold nanosol substrate, Sens Actuators B Chem. 255 (2018) 3187–3193.
[116] Z. Jiang, C. Li, Y. Liu, et al., A sensitive Galvanic replacement reaction-SERS method for Au(III) with *Victoria* blue B molecular probes in silver nanosol substrate, Sens Actuators B Chem. 251 (2017) 404–409.
[117] C. Li, Y. Qin, D. Li, et al., A highly sensitive enzyme catalytic SERS quantitative analysis method for ethanol with *Victoria* blue B molecular probe in the stable nanosilver sol substrate, Sens Actuators B Chem. 255 (2018) 3464–3471.
[118] K. Ponlamuangdee, C. Rattanabut, N. Viriyakitpattana, et al., Fabrication of paper-based SERS substrate using a simple vacuum filtration system for pesticides detection, Anal. Methods 14 (2022) 1765–1773.
[119] I. Shaikh, M.A. Haque, H. Pathan, et al., Spin-coated Ag NPs SERS substrate: Role of electromagnetic and chemical enhancement in trace detection of methylene blue and Congo red, Plasmonics 17 (2022) 1889–1900.
[120] P. Zhang, G. Liu, S. Feng, et al., Engineering of flexible granular Au nanocap ordered array and its surface enhanced Raman spectroscopy effect, Nanotechnology 31 (2020), 35303.

[121] R. Wang, Y. Xu, R. Wang, et al., A microfluidic chip based on an ITO support modified with Ag-Au nanocomposites for SERS based determination of melamine, Microchim. Acta 184 (2017) 279–287.
[122] K. Sivashanmugan, V.-H. Nguyen, B.-S. Nguyen, Tailoring a novel Au nanodot arrays on graphene substrate for a highly active surface-enhanced Raman scattering (SERS), Mater. Lett. 271 (2020), 127807.
[123] X. Zhang, S. Xu, S. Jiang, et al., Growth graphene on silver-copper nanoparticles by chemical vapor deposition for high-performance surface-enhanced Raman scattering, Appl. Surf. Sci. 353 (2015) 63–70.
[124] S. Zhang, J. Xu, Z. Liu, et al., Rapid and scalable preparation of flexible Ag nanoparticle-decorated nanocellulose SERS sensors by magnetron sputtering for trace detection of toxic materials, Cellulose 29 (2022) 9865–9879.
[125] C. Lai, G. Chen, L. Chen, et al., Gold nanoparticle-coated silicon cone array for surface-enhanced Raman spectroscopy, Spectrosc. Lett. 49 (2016) 51–55.
[126] H.Y. Wu, H.C. Lin, G.Y. Hung, et al., High sensitivity SERS substrate of a few nanometers single-layer silver thickness fabricated by DC magnetron sputtering technology, Nanomaterials 12 (2022), 2742.
[127] D. Han, B. Li, S. Yang, et al., Facile synthesis of Fe_3O_4@Au core-shell nanocomposites as an SERS substrate for the detection of thiram, Chem. J. Chin. Univ. 40 (2019) 2067–2074.
[128] Z. Wang, S. Zong, L. Wu, et al., SERS-activated platforms for immunoassay: Probes, encoding methods, and applications, Chem. Rev. 117 (2017) 7910–7963.
[129] J. Perumal, Y. Wang, A.B.E. Attia, et al., Towards a point-of-care SERS sensor for biomedical and agri-food analysis applications: A review of recent advancements, Nanoscale 13 (2021) 553–580.
[130] M. Barreiros dos Santos, L. Rodriguez-Lorenzo, R. Queirós, et al., Fundamentals of Biosensors and Detection Methods. Microfluidics and Biosensors in Cancer Research, Springer, 2022, pp. 3–29.
[131] S.S. Panikar, D. Cialla-May, E. De la Rosa, et al., Towards translation of surface-enhanced Raman spectroscopy (SERS) to clinical practice: Progress and trends, Trac Trends Anal. Chem. 134 (2021), 116122.
[132] H. Zhao, Y. Xu, C. Wang, et al., Design and fabrication of a microfluidic SERS chip with integrated Ag film@nanoAu, RSC Adv. 6 (2016) 14105–14111.
[133] I.J. Jahn, O. Žukovskaja, X.-S. Zheng, et al., Surface-enhanced Raman spectroscopy and microfluidic platforms: Challenges, solutions and potential applications, Analyst 142 (2017) 1022–1047.
[134] H. Samawi, D.-G. Chen, J. Yin, et al., Performance of diagnostic tests based on continuous bivariate markers, J. Appl. Stat. (2022) 1–18.
[135] J. Hua, L. Tian, Combining multiple biomarkers to linearly maximize the diagnostic accuracy under ordered multi-class setting, Stat. Methods Med. Res. 30 (2021) 1101–1118.
[136] Z. Liao, Y. Zhang, Y. Li, et al., Microfluidic chip coupled with optical biosensors for simultaneous detection of multiple analytes: A review, Biosens. Bioelectron. 126 (2019) 697–706.
[137] A.I. Saviñon-Flores, F. Saviñon-Flores, G. Trejo, et al., A review of cardiac troponin I detection by surface enhanced Raman spectroscopy: Under the spotlight of point-of-care testing, Front. Chem. 10 (2022), 1017305.
[138] R.A. Crocombe, Portable spectroscopy, Appl. Spectrosc. 72 (2018) 1701–1751.
[139] Y. Li, X. Liu, J. Guo, et al., Simultaneous detection of inflammatory biomarkers by SERS nanotag-based lateral flow assay with portable cloud Raman spectrometer, Nanomaterials 11 (2021), 1496.
[140] T. Mu, S. Li, H. Feng, et al., High-sensitive smartphone-based Raman system based on cloud network architecture, IEEE J. Sel. Top. Quantum Electron. 25 (2019) 1–6.
[141] F. Zeng, T. Mou, C. Zhang, et al., Paper-based SERS analysis with smartphones as Raman spectral analyzers, Analyst 144 (2018) 137–142.
[142] N. Bellassai, R. D'Agata, V. Jungbluth, et al., Surface plasmon resonance for biomarker detection: Advances in non-invasive cancer diagnosis, Front. Chem. 7 (2019), 570.
[143] H. Huang, Y. Yang, Y. Zhu, et al., Blood protein biomarkers in lung cancer, Cancer Lett. 551 (2022), 215886.
[144] H. Khan, M.R. Shah, J. Barek, et al., Cancer biomarkers and their biosensors: A comprehensive review, Trac Trends Anal. Chem. 158 (2023), 116813.
[145] N. Soda, K. Clack, M.J.A. Shiddiky, Recent advances in liquid biopsy technologies for cancer biomarker detection, Sens. Diagn. 1 (2022) 343–375.
[146] D. Mwanza, O. Adeniyi, S. Tesfalidet, et al., Capacitive label-free ultrasensitive detection of PSA on a covalently attached monoclonal anti-PSA antibody gold surface, J. Electroanal. Chem. 927 (2022), 116983.
[147] A. Usman, Nanoparticle enhanced optical biosensing technologies for prostate specific antigen biomarker detection, IEEE Rev. Biomed. Eng. 15 (2022) 122–137.
[148] G. Luo, K. Jin, S. Deng, et al., Roles of CA19-9 in pancreatic cancer: Biomarker, predictor and promoter, Biochim. Biophys. Acta Rev. Cancer 1875 (2021), 188409.
[149] H.J. Song, E.S. Yang, J.D. Kim, et al., Best serum biomarker combination for ovarian cancer classification, Biomed. Eng. Online 17 (2018), 152.
[150] J. Heylen, K. Punie, A. Smeets, et al., Elevated CA 15.3 in newly diagnosed breast cancer: A retrospective study, Clin. Breast Cancer 22 (2022) 579–587.
[151] J.D. Cohen, A.A. Javed, C. Thoburn, et al., Combined circulating tumor DNA and protein biomarker-based liquid biopsy for the earlier detection of pancreatic cancers, Proc. Natl. Acad. Sci. U S A 114 (2017) 10202–10207.
[152] A.L. Jones, L. Dhanapala, T.A. Baldo, et al., Prostate cancer diagnosis in the clinic using an 8-protein biomarker panel, Anal. Chem. 93 (2021) 1059–1067.
[153] G. Kumarasamy, G. Kaur, Protein biomarkers in gynecological cancers: The need for translational research towards clinical applications, Clin. Invest. Ginecol. Obstet. 49 (2022), 100735.
[154] L. Wu, X. Qu, Cancer biomarker detection: Recent achievements and challenges, Chem. Soc. Rev. 44 (2015) 2963–2997.
[155] H. Xiong, J. Yan, S. Cai, et al., Cancer protein biomarker discovery based on nucleic acid aptamers, Int. J. Biol. Macromol. 132 (2019) 190–202.
[156] K.L. Singampalli, J. Li, P.B. Lillehoj, Rapid magneto-enzyme-linked immunosorbent assay for ultrasensitive protein detection, Anal. Chim. Acta 1225 (2022), 340246.
[157] T. Chatterjee, A. Knappik, E. Sandford, et al., Direct kinetic fingerprinting and digital counting of single protein molecules, Proc. Natl. Acad. Sci. U S A 117 (2020) 22815–22822.
[158] Y.-T. Chen, L.-P. Tuan, H.-W. Chen, et al., Quantitative analysis of prostate specific antigen isoforms using immunoprecipitation and stable isotope labeling mass spectrometry, Anal. Chem. 87 (2015) 545–553.
[159] A. Zhang, C. Yin, Z. Wang, et al., Development and application of a fluorescence protein microarray for detecting serum alpha-fetoprotein in patients with hepatocellular carcinoma, J. Int. Med. Res. 44 (2016) 1414–1423.
[160] X. Min, S. Huang, C. Yuan, Dual-color quantum dots nanobeads based suspension microarray for simultaneous detection of dual prostate specific antigens, Anal. Chim. Acta 1204 (2022), 339704.
[161] Y. Yu, Q. Zhang, J. Buscaglia, et al., Quantitative real-time detection of carcinoembryonic antigen (CEA) from pancreatic cyst fluid using 3-D surface molecular imprinting, Analyst 141 (2016) 4424–4431.
[162] Y. Wang, Z. Zhang, V. Jain, et al., Potentiometric sensors based on surface molecular imprinting: Detection of cancer biomarkers and viruses, Sens Actuators B Chem. 146 (2010) 381–387.
[163] B.V. Chikkaveeraiah, A.A. Bhirde, N.Y. Morgan, et al., Electrochemical immunosensors for detection of cancer protein biomarkers, ACS Nano 6 (2012) 6546–6561.
[164] H.J. Lee, A.W. Wark, R.M. Corn, Microarray methods for protein biomarker detection, Analyst 133 (2008) 975–983.
[165] F. Zhou, M. Wang, L. Yuan, et al., Sensitive sandwich ELISA based on a gold nanoparticle layer for cancer detection, Analyst 137 (2012) 1779–1784.
[166] R.L. Woodbury, S.M. Varnum, R.C. Zangar, Elevated HGF levels in sera from breast cancer patients detected using a protein microarray ELISA, J. Proteome Res. 1 (2002) 233–237.
[167] Z. Cheng, N. Choi, R. Wang, et al., Simultaneous detection of dual prostate specific antigens using surface-enhanced Raman scattering-based immunoassay for accurate diagnosis of prostate cancer, ACS Nano 11 (2017) 4926–4933.
[168] W. Wang, W. Wang, L. Liu, et al., Nanoshell-enhanced Raman spectroscopy on a microplate for staphylococcal enterotoxin B sensing, ACS Appl. Mater. Interfaces 8 (2016) 15591–15597.
[169] H. Chon, C. Lim, S.M. Ha, et al., On-chip immunoassay using surface-enhanced Raman scattering of hollow gold nanospheres, Anal. Chem. 82 (2010) 5290–5295.
[170] B. Tang, J. Wang, J.A. Hutchison, et al., Ultrasensitive, multiplex Raman frequency shift immunoassay of liver cancer biomarkers in physiological media, ACS Nano 10 (2016) 871–879.
[171] X. Li, L. Li, Y. He, Preparation of a sandwich-like complex "MIPs-target molecule-magnetic SERS probe" and SERS determination of immunoglobulin G, Anal. Sci. 36 (2020) 1025–1030.
[172] W. Liang, H. Lin, J. Chen, et al., Utilization of nanoparticles in microfluidic systems for optical detection, Microsyst. Technol. 22 (2016) 2363–2370.
[173] E.E. Ahi, H. Torul, A. Zengin, et al., A capillary driven microfluidic chip for SERS based hCG detection, Biosens. Bioelectron. 195 (2022), 113660.
[174] R.K. Iles, P.J. Delves, S.A. Butler, Does hCG or hCGβ play a role in cancer cell biology? Mol. Cell. Endocrinol. 329 (2010) 62–70.
[175] J. Rodrigues, S.O. Pereira, N.F. Santos, et al., Insights on luminescence quenching of ZnO tetrapods in the detection of hCG, Appl. Surf. Sci. 527 (2020), 146813.
[176] R. Gao, Z. Cheng, A.J. deMello, et al., Wash-free magnetic immunoassay of the PSA cancer marker using SERS and droplet microfluidics, Lab Chip 16 (2016) 1022–1029.
[177] Z. Zheng, L. Wu, L. Li, et al., Simultaneous and highly sensitive detection of multiple breast cancer biomarkers in real samples using a SERS microfluidic chip, Talanta 188 (2018) 507–515.
[178] Q. Liao, C. Mu, D.-S. Xu, et al., Gold nanorod arrays with good reproducibility for high-performance surface-enhanced Raman scattering, Langmuir 25 (2009) 4708–4714.
[179] Q. Fu, Z. Zhan, J. Dou, et al., Highly reproducible and sensitive SERS substrates with Ag inter-nanoparticle gaps of 5 nm fabricated by ultrathin aluminum mask technique, ACS Appl. Mater. Interfaces 7 (2015) 13322–13328.
[180] W. Leng, P.J. Vikesland, Nanoclustered gold honeycombs for surface-enhanced Raman scattering, Anal. Chem. 85 (2013) 1342–1349.
[181] Y. Gu, Z. Li, S. Ge, et al., A microfluidic chip using Au@SiO_2 array-based highly SERS-active substrates for ultrasensitive detection of dual cervical cancer-related biomarkers, Anal. Bioanal. Chem. 414 (2022) 7659–7673.
[182] D. Cialla-May, X.S. Zheng, K. Weber, et al., Recent progress in surface-enhanced Raman spectroscopy for biological and biomedical applications: From cells to clinics, Chem. Soc. Rev. 46 (2017) 3945–3961.

[183] T. Hillig, P. Horn, A.B. Nygaard, et al., *In vitro* detection of circulating tumor cells compared by the CytoTrack and CellSearch methods, Tumour Biol. 36 (2015) 4597–4601.
[184] K. Jibin, R. Babu V, R.S. Jayasree, Graphene-gold nanohybrid-based surface-enhanced Raman scattering platform on a portable easy-to-use centrifugal prototype for liquid biopsy detection of circulating breast cancer cells, ACS Sustainable Chem. Eng. 9 (2021) 15496–15505.
[185] X. Xu, J. Lin, Y. Guo, et al., TiO_2-based surface-enhanced Raman scattering bio-probe for efficient circulating tumor cell detection on microfilter, Biosens. Bioelectron. 210 (2022), 114305.
[186] J. Loh, L. Jovanovic, M. Lehman, et al., Circulating tumor cell detection in high-risk non-metastatic prostate cancer, J. Cancer Res. Clin. Oncol. 140 (2014) 2157–2162.
[187] L. Dirix, A. Buys, S. Oeyen, et al., Circulating tumor cell detection: A prospective comparison between CellSearch® and RareCyte® platforms in patients with progressive metastatic breast cancer, Breast Cancer Res. Treat. 193 (2022) 437–444.
[188] E. Le Rhun, Q. Tu, M. De Carvalho Bittencourt, et al., Detection and quantification of CSF malignant cells by the CellSearch® technology in patients with melanoma leptomeningeal metastasis, Med. Oncol. 30 (2013), 538.
[189] X. Huang, P. Gao, Y. Song, et al., Meta-analysis of the prognostic value of circulating tumor cells detected with the CellSearch System in colorectal cancer, BMC Cancer 15 (2015), 202.
[190] S. Xu, L. Wu, Y. Qin, et al., Sequential ensemble-decision aliquot ranking isolation and fluorescence *in situ* hybridization identification of rare cells from blood by using concentrated peripheral blood mononuclear cells, Anal. Chem. 93 (2021) 3196–3201.
[191] J. Han, C. Lu, M. Shen, et al., Fast, reusable, cell uniformly distributed membrane filtration device for separation of circulating tumor cells, ACS Omega 7 (2022) 20761–20767.
[192] M. Aghaamoo, A. Aghilinejad, X. Chen, et al., On the design of deterministic dielectrophoresis for continuous separation of circulating tumor cells from peripheral blood cells, Electrophoresis 40 (2019) 1486–1493.
[193] A. Alazzam, B. Mathew, F. Alhammadi, Novel microfluidic device for the continuous separation of cancer cells using dielectrophoresis, J. Separ. Sci. 40 (2017) 1193–1200.
[194] S.P. Duffy, H. Ma, Morphological characteristics of CTCs and the potential for deformability-based separation. Z.H. Fan, Circulating Tumor Cells, John Wiley & Sons, Inc, Hoboken, 2016, pp. 147–172.
[195] G.E. Hvichia, Z. Parveen, C. Wagner, et al., A novel microfluidic platform for size and deformability based separation and the subsequent molecular characterization of viable circulating tumor cells, Int. J. Cancer 138 (2016) 2894–2904.
[196] S. He, J. Wei, L. Ding, et al., State-of-the-arts techniques and current evolving approaches in the separation and detection of circulating tumor cell, Talanta 239 (2022), 123024.
[197] L. Zhu, X. Feng, S. Yang, et al., Colorimetric detection of immunomagnetically captured rare number CTCs using mDNA-wrapped single-walled carbon nanotubes, Biosens. Bioelectron. 172 (2021), 112780.
[198] X. Wu, Z. Lin, C. Zhao, et al., Neutrophil membrane-coated immunomagnetic nanoparticles for efficient isolation and analysis of circulating tumor cells, Biosens. Bioelectron. 213 (2022), 114425.
[199] Z. Wang, Z. Wu, P. Ding, et al., Selective capture of circulating tumor cells by antifouling nanostructure substrate made of hydrogel nanoparticles, Colloids Surf. B Biointerfaces 202 (2021), 111669.
[200] X. Zhou, D. Bai, H. Yu, et al., Detection of rare CTCs by electrochemical biosensor built on quaternary PdPtCuRu nanospheres with mesoporous architectures, Talanta 253 (2023), 123955.
[201] S. Ju, C. Chen, J. Zhang, et al., Detection of circulating tumor cells: Opportunities and challenges, Biomark. Res. 10 (2022), 58.
[202] T. Palmela Leitão, M. Miranda, J. Polido, et al., Circulating tumor cell detection methods in renal cell carcinoma: A systematic review, Crit. Rev. Oncol. Hematol. 161 (2021), 103331.
[203] R.E. Wilson Jr., R. O'Connor, C.E. Gallops, et al., Immunomagnetic capture and multiplexed surface marker detection of circulating tumor cells with magnetic multicolor surface-enhanced Raman scattering nanotags, ACS Appl. Mater. Interfaces 12 (2020) 47220–47232.
[204] W. Xia, H. Li, Y. Li, et al., *In vivo* coinstantaneous identification of hepatocellular carcinoma circulating tumor cells by dual-targeting magnetic-fluorescent nanobeads, Nano Lett. 21 (2021) 634–641.
[205] L. Nie, F. Li, X. Huang, et al., Folic acid targeting for efficient isolation and detection of ovarian cancer CTCs from human whole blood based on two-step binding strategy, ACS Appl. Mater. Interfaces 10 (2018) 14055–14062.
[206] C. Wan, B. Zhou, Research progress on circulating tumor cells of hepatocellular carcinoma, J. Interv. Med. 4 (2021) 181–183.
[207] L. Zhu, H. Lin, S. Wan, et al., Efficient isolation and phenotypic profiling of circulating hepatocellular carcinoma cells via a combinatorial-antibody-functionalized microfluidic synergetic-chip, Anal. Chem. 92 (2020) 15229–15235.
[208] J.M. Park, J.Y. Lee, J.G. Lee, et al., Highly efficient assay of circulating tumor cells by selective sedimentation with a density gradient medium and microfiltration from whole blood, Anal. Chem. 84 (2012) 7400–7407.
[209] T. Mei, X. Lu, N. Sun, et al., Real-time quantitative PCR detection of circulating tumor cells using tag DNA mediated signal amplification strategy, J. Pharm. Biomed. Anal. 158 (2018) 204–208.
[210] J. Guo, B. Xiao, X. Zhang, et al., Combined use of positive and negative immunomagnetic isolation followed by real-time RT-PCR for detection of the circulating tumor cells in patients with colorectal cancers, J. Mol. Med. (Berl.) 82 (2004) 768–774.
[211] M. Guo, X. Li, S. Zhang, et al., Real-time quantitative RT-PCR detection of circulating tumor cells from breast cancer patients, Int. J. Oncol. 46 (2015) 281–289.
[212] F. Li, Q. Li, H. Zhou, et al., Detection of circulating tumor cells in breast cancer with a refined immunomagnetic nanoparticle enriched assay and nested-RT-PCR, Nanomed 9 (2013) 1106–1113.
[213] L. Hu, X. Chen, M. Chen, et al., Enrichment and detection of circulating tumor cells by immunomagnetic beads and flow cytometry, Biotechnol. Lett. 43 (2021) 25–34.
[214] Y. Lu, H. Liang, T. Yu, et al., Isolation and characterization of living circulating tumor cells in patients by immunomagnetic negative enrichment coupled with flow cytometry, Cancer 121 (2015) 3036–3045.
[215] T.G. Ntouroupi, S.Q. Ashraf, S.B. McGregor, et al., Detection of circulating tumour cells in peripheral blood with an automated scanning fluorescence microscope, Br. J. Cancer 99 (2008) 789–795.
[216] D.E. Sabath, M.E. Perrone, A. Clein, et al., Clinical validation of a circulating tumor cell assay using density centrifugation and automated immunofluorescence microscopy, Am. J. Clin. Pathol. 158 (2022) 270–276.
[217] Y. Chong, Y.C. Jung, E. Hwang, et al., Circulating tumor cell detection in lung cancer animal model, J. Chest Surg. 54 (2021) 460–465.
[218] X. Jie, M. Zhang, M. Du, et al., Detection of circulating tumor cells and evaluation of epithelial-mesenchymal transition patterns of circulating tumor cells in ovarian cancer, Transl. Cancer Res. 11 (2022) 2636–2646.
[219] R. Gao, C. Zhan, C. Wu, et al., Simultaneous single-cell phenotype analysis of hepatocellular carcinoma CTCs using a SERS-aptamer based microfluidic chip, Lab Chip 21 (2021) 3888–3898.
[220] Y. Xu, J. Lin, X. Wu, et al., A TiO_2-based bioprobe enabling excellent SERS activity in the detection of diverse circulating tumor cells, J. Mater. Chem. B 10 (2022) 3808–3816.
[221] P. Zhang, R. Zhang, M. Gao, et al., Novel nitrocellulose membrane substrate for efficient analysis of circulating tumor cells coupled with surface-enhanced Raman scattering imaging, ACS Appl. Mater. Interfaces 6 (2014) 370–376.
[222] C. Sun, R. Zhang, M. Gao, et al., A rapid and simple method for efficient capture and accurate discrimination of circulating tumor cells using aptamer conjugated magnetic beads and surface-enhanced Raman scattering imaging, Anal. Bioanal. Chem. 407 (2015) 8883–8892.
[223] S. Dey, R. Vaidyanathan, K.K. Reza, et al., A microfluidic-SERS platform for isolation and immuno-phenotyping of antigen specific T-cells, Sens Actuators B Chem. 284 (2019) 281–288.
[224] J. Lin, J. Zheng, A. Wu, An efficient strategy for circulating tumor cell detection: Surface-enhanced Raman spectroscopy, J. Mater. Chem. B 8 (2020) 3316–3326.
[225] X. Wu, L. Luo, S. Yang, et al., Improved SERS nanoparticles for direct detection of circulating tumor cells in the blood, ACS Appl. Mater. Interfaces 7 (2015) 9965–9971.
[226] Y. Pang, C. Wang, R. Xiao, et al., Dual-selective and dual-enhanced SERS nanoprobes strategy for circulating hepatocellular carcinoma cells detection, Chemistry 24 (2018) 7060–7067.
[227] J. Li, Y. Yuan, H. Gan, et al., Double-tetrahedral DNA probe functionalized Ag nanorod biointerface for effective capture, highly sensitive detection, and nondestructive release of circulating tumor cells, ACS Appl. Mater. Interfaces 14 (2022) 32869–32879.
[228] Z. Liu, R. Chen, Y. Li, et al., Integrated microfluidic chip for efficient isolation and deformability analysis of circulating tumor cells, Adv. Biosys. 2 (2018), 1800200.
[229] S.F. Berlanda, M. Breitfeld, C.L. Dietsche, et al., Recent advances in microfluidic technology for bioanalysis and diagnostics, Anal. Chem. 93 (2021) 311–331.
[230] Y. Zhang, Z. Wang, L. Wu, et al., Combining multiplex SERS nanovectors and multivariate analysis for *in situ* profiling of circulating tumor cell phenotype using a microfluidic chip, Small 14 (2018), e1704433.
[231] T.R. Szymborski, M. Czaplicka, A.B. Nowicka, et al., Dielectrophoresis-based SERS sensors for the detection of cancer cells in microfluidic chips, Biosensors 12 (2022), 681.
[232] J. Rezaie, T. Etemadi, M. Feghhi, The distinct roles of exosomes in innate immune responses and therapeutic applications in cancer, Eur. J. Pharmacol. 933 (2022), 175292.
[233] G. Babaei, M. Asghari Vostakolaei, M. Rajabi Bazl, et al., The role of exosomes in the molecular mechanisms of metastasis: Focusing on EMT and cancer stem cells, Life Sci. 310 (2022), 121103.
[234] P. Li, X. Yu, W. Han, et al., Ultrasensitive and reversible nanoplatform of urinary exosomes for prostate cancer diagnosis, ACS Sens. 4 (2019) 1433–1441.
[235] J. Liu, L. Ren, S. Li, et al., The biology, function, and applications of exosomes in cancer, Acta Pharm. Sin. B 11 (2021) 2783–2797.
[236] C. He, L. Li, L. Wang, et al., Exosome-mediated cellular crosstalk within the tumor

microenvironment upon irradiation, Cancer Biol. Med. 18 (2021) 21–33.
[237] Z. Tang, D. Li, S. Hou, et al., The cancer exosomes: Clinical implications, applications and challenges, Int. J. Cancer 146 (2020) 2946–2959.
[238] T.-D. Li, R. Zhang, H. Chen, et al., An ultrasensitive polydopamine bi-functionalized SERS immunoassay for exosome-based diagnosis and classification of pancreatic cancer, Chem. Sci. 9 (2018) 5372–5382.
[239] L.M. Channon, V.M. Tyma, Z. Xu, et al., Small extracellular vesicles (exosomes) and their cargo in pancreatic Cancer: Key roles in the hallmarks of cancer, Biochim. Biophys. Acta Rev. Cancer 1877 (2022), 188728.
[240] H. Mirzaei, A. Sahebkar, M.R. Jaafari, et al., Diagnostic and therapeutic potential of exosomes in cancer: The beginning of a new tale? J. Cell. Physiol. 232 (2017) 3251–3260.
[241] S.D. Ibsen, J. Wright, J.M. Lewis, et al., Rapid isolation and detection of exosomes and associated biomarkers from plasma, ACS Nano 11 (2017) 6641–6651.
[242] Y. Xiao, Y. Li, Y. Yuan, et al., The potential of exosomes derived from colorectal cancer as a biomarker, Clin. Chim. Acta 490 (2019) 186–193.
[243] F.Z. Farhana, M. Umer, A. Saeed, et al., Isolation and detection of exosomes using Fe_2O_3 nanoparticles, ACS Appl. Nano Mater. 4 (2021) 1175–1186.
[244] L. Zheng, H. Wang, P. Zuo, et al., Rapid on-chip isolation of cancer-associated exosomes and combined analysis of exosomes and exosomal proteins, Anal. Chem. 94 (2022) 7703–7712.
[245] K. Boriachek, M.K. Masud, C. Palma, et al., Avoiding pre-isolation step in exosome analysis: Direct isolation and sensitive detection of exosomes using gold-loaded nanoporous ferric oxide nanozymes, Anal. Chem. 91 (2019) 3827–3834.
[246] H. Xu, C. Liao, P. Zuo, et al., Magnetic-based microfluidic device for on-chip isolation and detection of tumor-derived exosomes, Anal. Chem. 90 (2018) 13451–13458.
[247] J. Chen, Y. Xu, Y. Lu, et al., Isolation and visible detection of tumor-derived exosomes from plasma, Anal. Chem. 90 (2018) 14207–14215.
[248] P. Zhang, M. He, Y. Zeng, Ultrasensitive microfluidic analysis of circulating exosomes using a nanostructured graphene oxide/polydopamine coating, Lab Chip 16 (2016) 3033–3042.
[249] L. Wang, M.-M. Pan, L. Xu, et al., Recent advances of emerging microfluidic chips for exosome mediated cancer diagnosis, Smart Mater. Med. 2 (2021) 158–171.
[250] P. Zhang, X. Zhou, Y. Zeng, Multiplexed immunophenotyping of circulating exosomes on nano-engineered ExoProfile chip towards early diagnosis of cancer, Chem. Sci. 10 (2019) 5495–5504.
[251] S. Hassanpour Tamrin, A. Sanati Nezhad, A. Sen, Label-free isolation of exosomes using microfluidic technologies, ACS Nano 15 (2021) 17047–17079.
[252] Z. Zhao, Y. Yang, Y. Zeng, et al., A microfluidic ExoSearch chip for multiplexed exosome detection towards blood-based ovarian cancer diagnosis, Lab Chip 16 (2016) 489–496.
[253] L. Zhao, H. Wang, J. Fu, et al., Microfluidic-based exosome isolation and highly sensitive aptamer exosome membrane protein detection for lung cancer diagnosis, Biosens. Bioelectron. 214 (2022), 114487.
[254] Q. Zhou, A. Rahimian, K. Son, et al., Development of an aptasensor for electrochemical detection of exosomes, Methods 97 (2016) 88–93.
[255] L. Kashefi-Kheyrabadi, J. Kim, S. Chakravarty, et al., Detachable microfluidic device implemented with electrochemical aptasensor (DeMEA) for sequential analysis of cancerous exosomes, Biosens. Bioelectron. 169 (2020), 112622.
[256] X. Lv, Z. Geng, Y. Su, et al., Label-free exosome detection based on a low-cost plasmonic biosensor array integrated with microfluidics, Langmuir 35 (2019) 9816–9824.
[257] C. Wang, C.H. Huang, Z. Gao, et al., Nanoplasmonic sandwich immunoassay for tumor-derived exosome detection and exosomal PD-L1 profiling, ACS Sens. 6 (2021) 3308–3319.
[258] D. Kwong Hong Tsang, T.J. Lieberthal, C. Watts, et al., Chemically functionalised graphene FET biosensor for the label-free sensing of exosomes, Sci. Rep. 9 (2019), 13946.
[259] W. Zhao, J. Hu, J. Liu, et al., Si nanowire Bio-FET for electrical and label-free detection of cancer cell-derived exosomes, Microsyst. Nanoeng. 8 (2022), 57.
[260] F. Song, C. Wang, C. Wang, et al., Enrichment-detection integrated exosome profiling biosensors promising for early diagnosis of cancer, Anal. Chem. 93 (2021) 4697–4706.
[261] S. Dong, Y. Wang, Z. Liu, et al., Beehive-inspired macroporous SERS probe for cancer detection through capturing and analyzing exosomes in plasma, ACS Appl. Mater. Interfaces 12 (2020) 5136–5146.
[262] X. Zhao, C. Luo, Q. Mei, et al., Aptamer-cholesterol-mediated proximity ligation assay for accurate identification of exosomes, Anal. Chem. 92 (2020) 5411–5418.
[263] Y. Wang, Q. Li, H. Shi, et al., Microfluidic Raman biochip detection of exosomes: A promising tool for prostate cancer diagnosis, Lab Chip 20 (2020) 4632–4637.
[264] Z. Han, X. Peng, Y. Yang, et al., Integrated microfluidic-SERS for exosome biomarker profiling and osteosarcoma diagnosis, Biosens. Bioelectron. 217 (2022), 114709.
[265] P. Mandel, P. Metais, Nuclear acids in human blood plasma, C. R. Seances Soc. Biol. Fil. 142 (1948) 241–243.
[266] S.A. Leon, B. Shapiro, D.M. Sklaroff, et al., Free DNA in the serum of cancer patients and the effect of therapy, Cancer Res. 37 (1977) 646–650.
[267] J. Bredno, O. Venn, X. Chen, et al., Circulating tumor DNA allele fraction: A candidate biological signal for multicancer early detection tests to assess the clinical significance of cancers, Am. J. Pathol. 192 (2022) 1368–1378.
[268] Y.K. Chae, M.S. Oh, Detection of minimal residual disease using ctDNA in lung cancer: Current evidence and future directions, J. Thorac. Oncol. 14 (2019) 16–24.
[269] A. Bent, S. Raghavan, A. Dasari, et al., The future of ctDNA-defined minimal residual disease: Personalizing adjuvant therapy in colorectal cancer, Clin. Colorectal Cancer 21 (2022) 89–95.
[270] S. Khakoo, A. Georgiou, M. Gerlinger, et al., Circulating tumour DNA, a promising biomarker for the management of colorectal cancer, Crit. Rev. Oncol. Hematol. 122 (2018) 72–82.
[271] M. Morais, D.M. Pinto, J.C. Machado, et al., ctDNA on liquid biopsy for predicting response and prognosis in locally advanced rectal cancer: A systematic review, Eur. J. Surg. Oncol. 48 (2022) 218–227.
[272] A. Campos-Carrillo, J.N. Weitzel, P. Sahoo, et al., Circulating tumor DNA as an early cancer detection tool, Pharmacol. Ther. 207 (2020), 107458.
[273] U. Malapelle, M. Buono, P. Pisapia, et al., Circulating tumor DNA in cancer: Predictive molecular pathology meets mathematics, Crit. Rev. Oncol. Hematol. 163 (2021), 103394.
[274] F.N. Al-Shaheri, M.S.S. Alhamdani, A.S. Bauer, et al., Blood biomarkers for differential diagnosis and early detection of pancreatic cancer, Cancer Treat. Rev. 96 (2021), 102193.
[275] L. Valihrach, P. Androvic, M. Kubista, Circulating miRNA analysis for cancer diagnostics and therapy, Mol. Aspect. Med. 72 (2020), 100825.
[276] F.M. Orlandella, A.E. De Stefano, M. Braile, et al., Unveiling the miRNAs responsive to physical activity/exercise training in cancer: A systematic review, Crit. Rev. Oncol. Hematol. 180 (2022), 103844.
[277] M. Bilal, A. Javaid, F. Amjad, et al., An overview of prostate cancer (PCa) diagnosis: Potential role of miRNAs, Transl. Oncol. 26 (2022), 101542.
[278] Y.-H. Zhang, M. Jin, J. Li, et al., Identifying circulating miRNA biomarkers for early diagnosis and monitoring of lung cancer, Biochim. Biophys. Acta Mol. Basis Dis. 1866 (2020), 165847.
[279] Z. Chong, S.K. Yeap, W.Y. Ho, Roles of circulating microRNA(s) in human breast cancer, Arch. Biochem. Biophys. 695 (2020), 108583.
[280] D. Kashyap, H. Kaur, Cell-free miRNAs as non-invasive biomarkers in breast cancer: Significance in early diagnosis and metastasis prediction, Life Sci. 246 (2020), 117417.
[281] M.A. Mori, R.G. Ludwig, R. Garcia-Martin, et al., Extracellular miRNAs: From biomarkers to mediators of physiology and disease, Cell Metabol. 30 (2019) 656–673.
[282] M.P. Dragomir, E. Knutsen, G.A. Calin, Classical and noncanonical functions of miRNAs in cancers, Trends Genet. 38 (2022) 379–394.
[283] G.M. Dar, S. Agarwal, A. Kumar, et al., A non-invasive miRNA-based approach in early diagnosis and therapeutics of oral cancer, Crit. Rev. Oncol. Hematol. 180 (2022), 103850.
[284] V. De Guire, R. Robitaille, N. Tétreault, et al., Circulating miRNAs as sensitive and specific biomarkers for the diagnosis and monitoring of human diseases: Promises and challenges, Clin. Biochem. 46 (2013) 846–860.
[285] A. Ismail, H.A. El-Mahdy, A.I. Abulsoud, et al., Beneficial and detrimental aspects of miRNAs as chief players in breast cancer: A comprehensive review, Int. J. Biol. Macromol. 224 (2023) 1541–1565.
[286] K.N. Seale, K.H.R. Tkaczuk, Circulating biomarkers in breast cancer, Clin. Breast Cancer 22 (2022) e319–e331.
[287] J. Zhang, H. Zhao, Y. Gao, et al., Secretory miRNAs as novel cancer biomarkers, Biochim. Biophys. Acta Rev. Cancer 1826 (2012) 32–43.
[288] N.G. Elhelbawy, I.F. Zaid, A.A. Khalifa, et al., *miRNA-148a* and *miRNA-30c* expressions as potential biomarkers in breast cancer patients, Biochem. Biophys. Rep. 27 (2021), 101060.
[289] M.A. Abbas, I.E.T. El Sayed, A.M. Kamel Abdu-Allah, et al., Expression of *miRNA-29b* and *miRNA-31* and their diagnostic and prognostic values in Egyptian females with breast cancer, Noncoding RNA Res. 7 (2022) 248–257.
[290] A. Adam-Artigues, I. Garrido-Cano, S. Simón, et al., Circulating *miR-30b-5p* levels in plasma as a novel potential biomarker for early detection of breast cancer, ESMO Open 6 (2021), 100039.
[291] H. Si, X. Sun, Y. Chen, et al., Circulating *microRNA-92a* and *microRNA-21* as novel minimally invasive biomarkers for primary breast cancer, J. Cancer Res. Clin. Oncol. 139 (2013) 223–229.
[292] H.J. Seok, Y.E. Choi, J.Y. Choi, et al., Novel *miR-5088-5p* promotes malignancy of breast cancer by inhibiting DBC2, Mol. Ther. Nucleic Acids 25 (2021) 127–142.
[293] J. Wei, Y. Wang, J. Gao, et al., Detection of $BRAF^{V600E}$ mutation of thyroid cancer in circulating tumor DNA by an electrochemical-enrichment assisted ARMS-qPCR assay, Microchem. J. 179 (2022), 107452.
[294] J. Yin, L. Xia, Z. Zou, et al., A direct and multiplex digital PCR chip for *EGFR* mutation, Talanta 250 (2022), 123725.
[295] Z. Geng, S. Li, L. Zhu, et al., "Sample-to-answer" detection of rare ctDNA mutation from 2 mL plasma with a fully integrated DNA extraction and digital droplet PCR microdevice for liquid biopsy, Anal. Chem. 92 (2020) 7240–7248.
[296] H. Yuan, W. Gao, J. Yin, et al., Detection of *EGFR* gene with a droplet digital PCR chip integrating a double-layer glass reservoir, Anal. Biochem. 656 (2022), 114877.

[297] C. Hu, L. Zhang, Z. Yang, et al., Graphene oxide-based qRT-PCR assay enables the sensitive and specific detection of miRNAs for the screening of ovarian cancer, Anal. Chim. Acta 1174 (2021), 338715.
[298] T. Yoshinami, N. Kagara, D. Motooka, et al., Detection of ctDNA with personalized molecular barcode NGS and its clinical significance in patients with early breast cancer, Transl. Oncol. 13 (2020), 100787.
[299] M.A. Emelyanova, E.N. Telysheva, K.V. Orlova, et al., Microarray-based analysis of the *BRAF V600* mutations in circulating tumor DNA in melanoma patients, Cancer Genet. 250–251 (2021) 25–35.
[300] F. Damin, S. Galbiati, N. Soriani, et al., Analysis of *KRAS, NRAS* and *BRAF* mutational profile by combination of in-tube hybridization and universal tag-microarray in tumor tissue and plasma of colorectal cancer patients, PLoS One 13 (2018), e0207876.
[301] N. Nishida, M. Nagahara, T. Sato, et al., Microarray analysis of colorectal cancer stromal tissue reveals upregulation of two oncogenic miRNA clusters, Clin. Cancer Res. 18 (2012) 3054–3070.
[302] W. Ma, L. Liu, X. Zhang, et al., A microfluidic-based SERS biosensor with multifunctional nanosurface immobilized nanoparticles for sensitive detection of microRNA, Anal. Chim. Acta 1221 (2022), 340139.
[303] J. Dai, C. Xing, Y. Lin, et al., Localized DNA catalytic hairpin assembly reaction on DNA origami for tumor-associated microRNA detection and imaging in live cells, Sens Actuators B Chem. 344 (2021), 130195.
[304] L. Li, X. Fang, J. Le, et al., Highly sensitive detection and intracellular imaging of microRNAs based on target-triggered cascade catalytic hairpin assembly, Talanta 250 (2022), 123753.
[305] M. Yang, H. Li, X. Li, et al., Catalytic hairpin self-assembly regulated chameleon silver nanoclusters for the ratiometric detection of CircRNA, Biosens. Bioelectron. 209 (2022), 114258.
[306] X. Cao, Y. Mao, Y. Gu, et al., Highly sensitive and simultaneous detection of ctDNAs related to non-small cell lung cancer in serum using a catalytic hairpin assembly strategy in a SERS microfluidic chip, J. Mater. Chem. B 10 (2022) 6194–6206.
[307] X. Cao, S. Ge, X. Zhou, et al., A dual-signal amplification strategy based on pump-free SERS microfluidic chip for rapid and ultrasensitive detection of non-small cell lung cancer-related circulating tumour DNA in mice serum, Biosens. Bioelectron. 205 (2022), 114110.
[308] Y. Gu, D. Cao, Y. Mao, et al., A SERS microfluidic chip based on hpDNA-functioned Au-Ag nanobowl array for efficient simultaneous detection of non-small cell lung cancer-related microRNAs, Microchem. J. 182 (2022), 107836.
[309] Z. Wang, S. Zong, Z. Wang, et al., Microfluidic chip based micro RNA detection through the combination of fluorescence and surface enhanced Raman scattering techniques, Nanotechnology 28 (2017), 105501.
[310] R. Lampignano, V. Kloten, T. Krahn, et al., Integrating circulating miRNA analysis in the clinical management of lung cancer: Present or future? Mol. Aspect. Med. 72 (2020), 100844.
[311] V.D. Phung, W.S. Jung, T.A. Nguyen, et al., Reliable and quantitative SERS detection of dopamine levels in human blood plasma using a plasmonic Au/Ag nanocluster substrate, Nanoscale 10 (2018) 22493–22503.
[312] Y. Wang, C. Yu, H. Ji, et al., Label-free therapeutic drug monitoring in human serum by the 3-step surface enhanced Raman spectroscopy and multivariate analysis, Chem. Eng. J. 452 (2023), 139588.
[313] Y. Zhang, Y. Zou, F. Liu, et al., Stable graphene-isolated-Au-nanocrystal for accurate and rapid surface enhancement Raman scattering analysis, Anal. Chem. 88 (2016) 10611–10616.
[314] Y. Wang, M. Guan, C. Hu, et al., High-sensitivity biosensor based on SERS integrated with dendrimer-assisted boronic acid-functionalized magnetic nanoparticles for IL-6 detection in human serum, Nanotechnology 34 (2023), 355701.
[315] K. Chen, H. Chen, S. Liang, et al., A background-free SERS strategy for sensitive detection of hydrogen peroxide, Molecules 27 (2022), 7918.
[316] D. Zhu, A. Li, Y. Di, et al., Interference-free SERS nanoprobes for labeling and imaging of MT1-MMP in breast cancer cells, Nanotechnology 33 (2021), 115702.
[317] K.W. Kho, U.S. Dinish, A. Kumar, et al., Frequency shifts in SERS for biosensing, ACS Nano 6 (2012) 4892–4902.
[318] W. Zhu, J.A. Hutchison, M. Dong, et al., Frequency shift surface-enhanced Raman spectroscopy sensing: An ultrasensitive multiplex assay for biomarkers in human health, ACS Sens. 6 (2021) 1704–1716.
[319] H. Ma, X. Sun, L. Chen, et al., Multiplex immunochips for high-accuracy detection of AFP-L3% based on surface-enhanced Raman scattering: Implications for early liver cancer diagnosis, Anal. Chem. 89 (2017) 8877–8883.
[320] L. Wang, M. Pumera, Recent advances of 3D printing in analytical chemistry: Focus on microfluidic, separation, and extraction devices, Trac Trends Anal. Chem. 135 (2021), 116151.
[321] J. Parisi, Q. Dong, Y. Lei, *In situ* microfluidic fabrication of SERS nanostructures for highly sensitive fingerprint microfluidic-SERS sensing, RSC Adv. 5 (2015) 14081–14089.