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Review article

SERS sensing for cancer biomarker: Approaches and directions

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ABSTRACT

These days, cancer is thought to be more than just one illness, with several complex subtypes that require different screening approaches. These subtypes can be distinguished by the distinct markings left by metabolites, proteins, miRNA, and DNA. Personalized illness management may be possible if cancer is categorized according to its biomarkers. In order to stop cancer from spreading and posing a significant risk to patient survival, early detection and prompt treatment are essential. Traditional cancer screening techniques are tedious, time-consuming, and require expert personnel for analysis. This has led scientists to reevaluate screening methodologies and make use of emerging technologies to achieve better results. Using time and money saving techniques, these methodologies integrate the procedures from sample preparation to detection in small devices with high accuracy and sensitivity. With its proven potential for biomedical use, surface-enhanced Raman scattering (SERS) has been widely used in biosensing applications, particularly in biomarker identification. Consideration was given especially to the potential of SERS as a portable clinical diagnostic tool. The approaches to SERS-based sensing technologies for both invasive and non-invasive samples are reviewed in this article, along with sample preparation techniques and obstacles. Aside from these significant constraints in the detection approach and techniques, the review also takes into account the complexity of biological fluids, the availability of biomarkers, and their sensitivity and selectivity, which are generally lowered. Massive ways to maintain sensing capabilities in clinical samples are being developed recently to get over this restriction. SERS is known to be a reliable diagnostic method for treatment judgments. Nonetheless, there is still room for advancement in terms of portability, creation of diagnostic apps, and interdisciplinary AI-based applications. Therefore, we will outline the current state of technological maturity for SERS-based cancer biomarker detection in this article. The review will meet the demand for reviewing various sample types (invasive and non-invasive) of cancer biomarkers and their detection using SERS. It will also shed light on the growing body of research on portable methods for clinical application and quick cancer detection.

1. Introduction

Cancer is a global health issue, approximately 10 million cancer-related casualties are recorded worldwide. The World Health Organization (WHO) estimated that by 2040, it could be crossing over 28.9 million new cancer cases annually. An early-stage cancer diagnosis is one of the medical and research-highlighted questions of general practice. A rapid path of cancer screening supports doctors to advise for

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the therapy as per the marker detection. This makes primary care an important area for biomedical research to provide a robust solution. Regardless of the significance of a precise diagnosis of cancer, some patients still suffer from diagnostic errors. An accurate and complete diagnosis is critical during cancer care. The rate of diagnostic error is 1–6%, depending on factors such as anatomic site [1]. The present diagnostic screening comes with certain working constraints (e.g., a diagnostic test with a false-positive or false-negative result). Molecular tests for forecasting cancer with a molecular precision aim to ensure the delivery of the rapid target-specific diagnostic of abnormalities in a patient's cancer. It is important to note that biopsy was once the primary method in pathology for accurate diagnosis, but nowadays significantly greater confidence is generated in molecular testing [2]. This molecular testing provides cancer diagnostic and distinct subtypes of various cancers [3]. The early diagnostics and interpretation of biorecognition molecules involve a broad spectrum of analysis of biomolecules including nucleic acids, proteins, etc. Any alteration in these molecules can lead to distinct diseases at the molecular level. These biomolecular changes are ideal diagnostic biomarkers and therapeutic targets in the early diagnosis of the disease. Therefore, identifying specific recognizing strategies with multiplexing potential is crucial for biomolecular analysis and disease stratification. Specifically for cancer screening it is important to distinguish the place of illness to assist in investigation or direct attention based on symptoms. Biomarker screening offers precise screening and pre-information even when patients are asymptomatic. The early recognition of cancer biomarkers (nucleic acids, proteins, lipids, metabolites, etc.) within non-invasive blood/urine is an encouraging step for early-stage cancer screening. Importantly, the best screening biomarker requires precision, must have great sensitivity for very low sample concentrations and be easily accessible for screening [4]. Usually, the aggressive type of cancer is having very short duration of detection due to its high spreadability and might need higher precision. The overall requirement for cancer screening is to have a specific biomarker as well as advanced techniques with high precision.

Various sensing technologies including electrochemical, fluorescence, and molecular PCR-based detection have been investigated for decades, but most of them still lack either precision or sensitivity to reach the threshold [5]. Over the development, SERS has evolved as an extremely capable tool for multiplex bioanalysis and a potentially ultimate option for clinical diagnostics [6]. This fulfills the requirement for precision in detection. Since SERS provides a fingerprint for each analyte showing narrow and sharp emission peaks, excellent sensitivity and allows discrimination between samples [7]. The appropriateness of SERS for operative biomedical applications twigs between flexible experimental conditions and metallic nanostructures as signal-enhancing platforms. Precisely, SERS signals are molecularly individual and discernible, therefore multiplex identification of biomarkers in complex biological mixes is possible. Despite progress in the technical aspects of multiplex SERS analysis, there is still a large gap in the transformation of SERS technology for diagnosis applications. With the growing advance towards precision medicine, SERS is a promising multiplex technique for precise biomarker recognition and rapid and economical disease stratification in clinical practice. Despite the numerous advantages and after decades of advanced research published about SERS detection, the scientist is still at the demonstrative concept stage. The clinical SERS applications and laboratory-based procedures require to be progressively developed.

According to recent biosensing research, microfluidic chips have emerged as the most popular and extensively utilized method for precisely preparing and treating detection samples. Achieving accuracy and sensitivity for SERS detection methods is also significantly influenced by it [8]. Its low-volume manipulation of the sample is very important in cancer biomarkers where the sample volume is relatively small. Microfluidic technology also comes with great flexibility and potential for the design of novel approaches for cancer screening and treatment. Though microfluidic-based sensing platforms are not yet very popular and pass clinical requirements to be used in hospitals, they are still under various research to be used in diagnostics. With the potential of flexibility in microfluidic approaches, it demonstrates the most sensitivity and accuracy for screening cancer biomarkers and therapeutic approaches over conventional assays. Microfluidic lab-on-a-chip is a novel approach for cancer detection as well as for expansion toward new drug delivery and model systems for cancer treatment. Various approaches, including microfluidic chips, have become popular for the detection of cancer due to the significant and reliable source of blood biomarkers [9]. Liquid biopsies are traditionally used for detecting cancer biomarkers in body fluids that assist in cancer screening. The biomarker analytes are subdivided into two types: invasive and non-invasive. The blood biopsy is largely used because this method is a minimally invasive procedure and provides prominent biomarkers including metabolic markers, circulating tumor cells (CTCs), protein biomarkers on cells, etc., schematically presented in Fig. 1. Several detection methods are developed based on the specific recognition of intracellular biomarkers, or the cell membrane biomarkers. Traditionally, in-vivo and in-vitro experimental versions are applied in therapeutics for cancer [10]. Herein, we take a short step toward associated SERS clinical translation challenges, specifically: label-based SERS tags, label-free SERS strategy, and clinical validation. This review will explore challenges and intends prospective solutions to fill the multiplex SERS biomolecular analysis gap from lab-to-clinic.

2. SERS sensing strategies

SERS is a powerful analytical tool for the detection of cancer biomarkers due to its high sensitivity, specificity, and ability to detect molecular information from biological samples [11,12]. SERS is a Raman scattering spectroscopy and therefore a non-destructive vibrational technique based on the inelastic scattering of monochromatic light by molecules [13]. The Raman effect takes place when a molecule is illuminated by a laser beam and part of the incident light is absorbed exciting the molecule to a virtual energy level [14,15]. Rapidly, the molecule falls back to a permitted level and the adsorbed photons are scattered majority with the same frequency (an elastic interaction known as Rayleigh scattering) but a very small fraction (1 in 10^6-10^8 photons depending on the sample) are inelastically scattered, known as Raman scattering. When the frequency of the scattered photons is lower than the incident energy (energy transfer from the laser to the molecule) is known as Stokes scattering while when the frequency is higher (energy transfer from the molecule to the scattered photon) is known as anti-Stokes scattering. Normally, anti-Stockes scattering is less frequent than Stokes due to the lower number of molecules that populate the excited vibrational levels. The energy transfer between the laser and the molecule corresponds with the vibrational energy levels of the molecule, due to this the resulting Raman spectrum contains information about the molecular vibrations and rotational modes of the molecule, resulting in a unique fingerprint characteristic for each molecule [16]. Raman spectroscopy is widely used to identify chemical compounds, determine their concentrations, and provide information about their structure and conformation. However, the major limitation of Raman spectroscopy is the weakness of the signals, which limits its application. It is noteworthy that the Raman signals of a certain molecule can be substantially enhanced when located close to or near an SERS platform [17]. This augmentation of Raman signals from molecules is attributed to various mechanisms, with the electromagnetic and chemical pathways emerging as the most widely accepted [18–20].The electromagnetic mechanism is intricately dependent on the localized surface plasmon resonance excitation of plasmonic nanostructures which generates highly intense electromagnetic fields. On the contrary, the chemical mechanism stems from distinct interactions between the target molecules and the SERS substrate, encompassing the exchange of electrons, either through sharing or transferring with the nanostructure. Therefore, SERS technique offers the benefits of Raman spectroscopy while capitalizing on

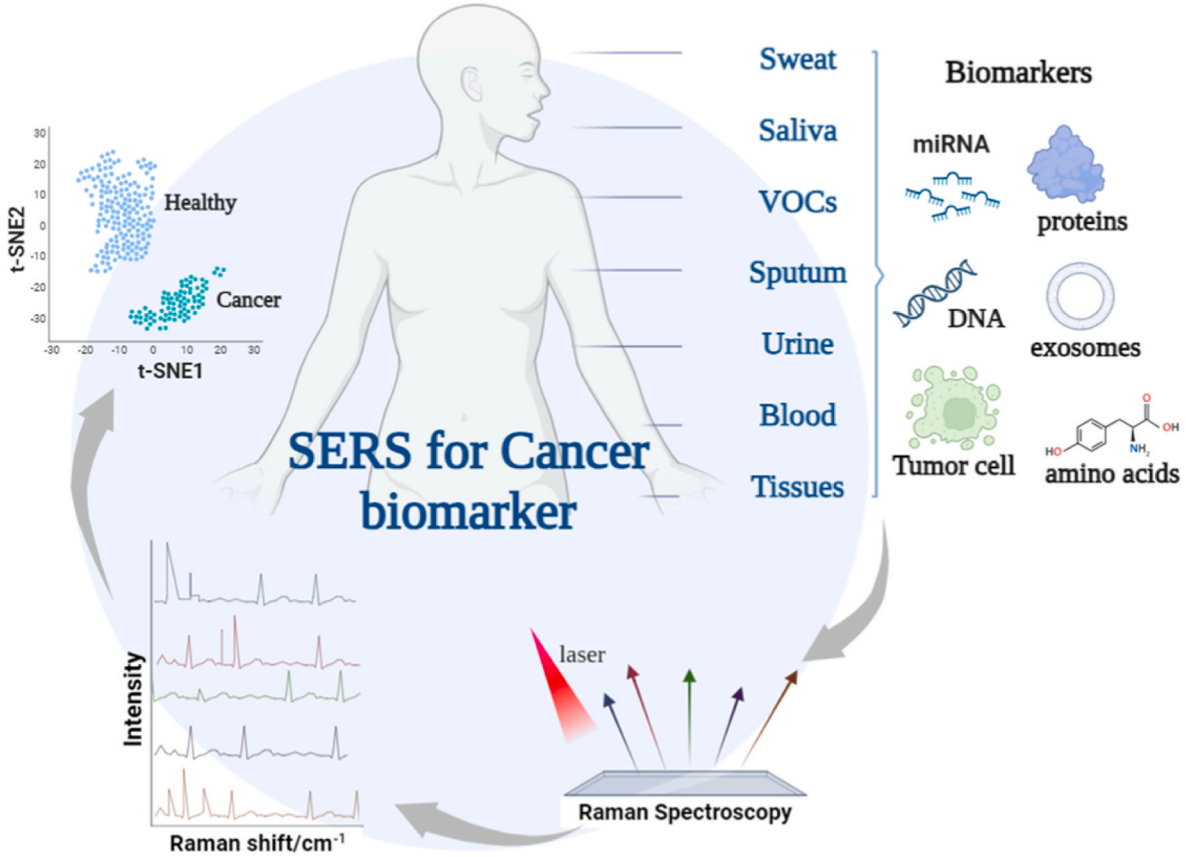


Fig. 1. A schematic presentation of various biomarkers present in non-invasive and invasive type samples used in SERS detection approaches for cancer screening.

the amplification of the Raman signal through the interaction between the analyte molecule and the nanostructured platform [21]. When dealing with noble metal nanostructures, the amplification of the Raman signal observed in a SERS spectrum is mainly due to the electromagnetic field generated at the metal surfaces (electromagnetic enhancement) but also due to the electron transfer process occurring between the analyte and metal nanostructures [22]. The SERS enhancement factor can be as high as 10^{14}, which even enables the detection of single molecules [23–26]. SERS brings several advantages over traditional diagnostic techniques for cancer biomarker detection. It is a sensitive technique that can distinguish trace amounts of analytes even in complex biological samples like blood, urine, and tissue, which is essential for the early recognition of cancer [27]. Besides, SERS is highly chemical specific and can differentiate between molecules that have similar structures but different functional groups, which is important for identifying cancer biomarkers from a particular type of cancer. Furthermore, its elevated specificity together with the narrowness of the peaks permits multiplex detection of cancer biomarkers [28].

Despite its potential, the application of SERS for the discovery of cancer biomarkers still faces several challenges. One of the main challenges deals with the reproducibility and standardization of the SERS signal, which is closely related to the performance of SERS substrates. The most common SERS platforms are based on the employment of noble metal nanoparticles, usually gold and silver nanoparticles due to their unique optical properties in the visible-NIR region. In this case, factors such as nanoparticle size, shape and composition, interparticle distance or assembly play crucial roles in determining the SERS performance [29–31]. Also, the nanoparticle spatial distribution within the substrate is very important to improve SERS enhancement, especially attending to the formation of hot-spots that increase the SERS enhancement of the substrate when the target molecule is situated in their junctions [32–34].

Apart from gold and silver, other cheaper metals such as aluminum or copper have also been employed for SERS sensing, although their limited sensitivity and stability have made them less appealing thus far [35,36]. In the field of biomedicine, despite silver's higher efficiency compared to gold, the prevalent use of gold in SERS applications is attributed to the lower chemical stability of silver. Furthermore, more recently non noble metal nanostructures have been employed to fabricate SERS substrates. These materials show several advantages, including lower cost, ease of large-scale preparation, high reproducibility, tunable electronic structures, high carrier mobility, chemical inertness, and flexibility [37,38]. In contrast to plasmonic materials, where SERS enhancement is mainly driven by the electromagnetic effect, these materials predominantly leverage the chemical effect. Therefore, their SERS efficiency is typically lower than that of noble metal nanostructures. Interestingly, the synergistic combination of noble metal platforms with non-noble metal materials could help to overcome their limitations and enhances the detection performance. However, this field is still in its early stages, and there is considerable progress yet to be made [39]. In conclusion, the development of novel nanoarchitectures that maximize SERS sensitivity by increasing the electric field strength or enhancing chemical interactions with the target analyte through chemical mechanisms, while exhibiting stability and reproducibility, is key. Numerous review reports have extensively examined the design and fabrication of SERS substrates [38–41].

Another crucial concern involves crafting SERS substrates that are both reliable and sensitive, capable of amplifying the Raman signal while mitigating background noise. This is particularly challenging when dealing with biological samples, given the high complexity of the medium. In this sense will be essential the progress in artificial intelligence (AI) and machine learning techniques which will allow us to reach lower limit of detection, multiplex detection, or faster data analysis. In the recognition of cancer biomarkers, the SERS performance primarily

depends on the efficient capture of biomarkers by surface-grafted agents, facilitating their localization on SERS platforms. Two distinct methodologies have been developed for biomarker detection: label-free or direct SERS, and SERS tag or indirect SERS. Both strategies will be discussed in the following sections.

2.1. SERS for direct detection strategy

Label-free SERS methodology is based on the detection of analytes without the use of any labels or probes, allowing to obtain directly qualitative and quantitative information about the target molecule (Fig. 2A) [42,43]. This method relies on the intrinsic properties of the molecule to interact with the plasmonic surface and generate a SERS signal. Label-free SERS methodology has several advantages over traditional labeling methods, including simplicity, speed, and the ability to detect a wide range of analytes [44]. However, due to the high dependence between the SERS signal within the analyte-metal surface distance [45], a critical issue is the accessibility/affinity of the analyte to the metal surface. For instance, another disadvantage to applying this methodology to real-life samples is the presence of other non-interest molecules that can interfere with the SERS measurement and result in complex data. Fortunately, some classical statistical methods such as principal components analysis (PCA) [46–48], multivariate analysis [49], and, more recently, machine learning too [50–55] allow for separating and differentiating the information from the target analyte considering the full spectral fingerprint. These statistical tools will be of significant relevance when transitioning to the detection of real samples, enabling accurate identification of target analytes even in complex media as well as multiplex detection and quantification of target analytes.

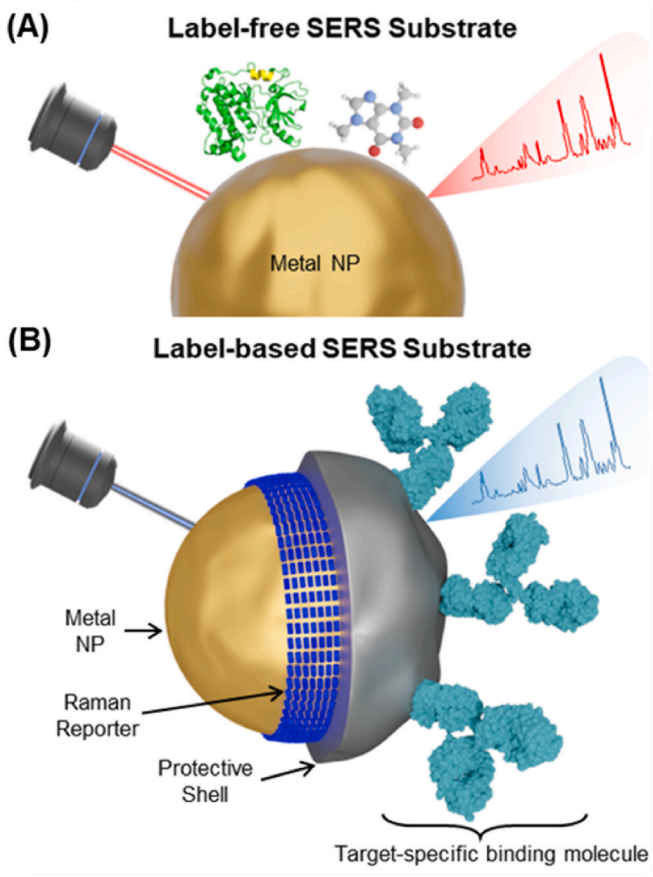


Fig. 2. Schematic illustration of the direct (label-free) and indirect (label-based) SERS detection. (A) The direct SERS for the detection of any RAMAN active molecule that interacts with the plasmonic metal surface. (B) The indirect SERS for the detection of the target molecule when it interacts with the binding part of the SERS tag thanks to the plasmonic metal nanoparticle functionalized with a Raman reporter molecule.

Researchers have previously employed the label-free approach to identify CTCs in the bloodstream of cancer patients, aiming to detect a wide range of cancer biomarkers such as nucleic acids, proteins, and small molecules [48,49]. CTCs, which originate from primary tumors, hold promise as valuable indicators for cancer diagnosis and surveillance. For instance, this approach has shown promise in identifying prostate cancer subtypes and predicting hepatocellular carcinoma by analyzing distinctive Raman peaks.

2.2. SERS for indirect detection strategy

On the other hand, indirect SERS detection of biomarkers typically involves the detection of a SERS tag capable of specifically recognizing and binding to the target biomarker [42,56]. The SERS tag is a structure (Fig. 2B) formed by a metal nanoparticle (typically gold or silver) that enhances the SERS signal of a Raman reporter bound directly to the metal surface, and a target-specific binding molecule responsible for recognizing and binding selectively to the target analyte [28,57,58]. Sometimes, the SERS tags also can include a protective shell to enhance the colloidal stability, avoid desorption of the Raman reporter or facilitate the functionalization of the metal nanoparticles with the targeting molecule [59]. For instance, this indirect method has been used to subtype prostate cancer tumors by a multiplexed platform strategy [60]. In this approach, 5 different and distinguishable SERS-Tag were prepared by conjugating individually five different PCa (prostate cancer???) amplicons with gold nanoparticles previously functionalized with different Raman probe molecules. In another work, Y. Zhang and co-workers developed SERS-tags constituted of Au–Ag/Au core-shell nanorod with an intra-nanoparticle gap containing a Raman reporter and functionalized with certain aptamers for the specific detection of MCF-7 (Michigan Cancer Foundation), breast cancer cell line in a blood mimicking fluid [61]. The main advantage of this methodology is that SERS tags are not affected by the cross-section of the target molecule or its affinity for the metal surface, as opposed to label-free methodology. However, the fabrication of SERS tags can be a laborious process and they are specific to binding to a particular target, which limits their application compared to label-free systems.

3. Biomarkers and strategies

Biomarkers are defined as “a characteristic that is measured as an indicator of normal biological processes, pathogenic processes or responses to an exposure or intervention” [62]. It is very important to find a biomarker that can be measured precisely, reproducibly and reliably. Often, bioassays are not validated because of the complexity of the validation process. The significance of specific diagnostic biomarkers will change as measurement techniques advance. Cancer biomarkers are present in tumor tissues, serum, and urine, and include a wide variety of molecules such as different kinds of proteins: transcription factors cell surface receptors, DNA, mRNA, enzymes, and metabolites [63]. Measuring biomarkers from samples obtained in a minimally invasive manner has the potential to significantly increase accessibility. It is important to find biomarkers with sufficient sensitivity (detect true positives) and specificity (detect true negatives) for cancer diagnostics. New technologies such as SERS and analytical tools such as machine learning are beginning to expand the possibilities of employing these biomarkers as targets for cancer diagnosis.

3.1. Biomarkers in non-invasive sample

Non-invasive samples are easy and quick to obtain and can be stored for long periods, have therefore been increasingly used in clinical

practice and screening programs in recent years. The gold standard microscopic imaging approach is an important methodology in cancer diagnosis and staging. Prostate, breast, and lung cancer types are usually classified and/or identified by the combination of imaging techniques and machine learning methods, which allows for diagnosing patients in a short time, in a cost-effective way. Additionally, samples can be recollected many times, on any patient, on-site, which helps in effective diagnostic. Access to the sample is also possible even at remote sites, where access to sophisticated equipment and clinical expertise are not easily available. The non-invasive biomarkers also have a series of advantages and disadvantages as listed in Table 1.

Depending on the type of body fluid, the detection of biomarkers may not require special equipment or specific training. The detection of circulating biomarkers can be performed with fast and cost-effective assays with high specificity and sensitivity. Sensitive methods, such as SERS, have been studied for their potential use in clinical practices for the early detection of cancer and monitoring of treatments through the search for biomarkers in non-invasive samples. This approach has demonstrated promising and highly accurate results for various tumor types, including lung cancer, prostate cancer, and gastric cancer [64–66]. However, analyzing biomarkers in liquid biopsies can be challenging due to the low number of molecules, such as cell-free nucleic acids and exosomes, as well as biological variability [67]. The different non-invasive biomarkers can be classified according to the origin of the sample: sweat, saliva, sputum, urine, and volatile organic compounds (VOCs). Some examples of non-invasive biomarkers are listed in Table 2.

3.1.1. Cancer biomarker in sweat

Sweat is a pain-free human biofluid that can be easily collected, rendering it an interesting biofluid for SERS applications (see Table 3). Sweat is not commonly used for cancer detection, but in this fluid creatinine and cortisol levels [68], drugs [69], and proteins [70] can be analyzed. Interestingly, sweat samples can be analyzed by SERS without any purification or processing. Nevertheless, obtaining a large amount of fluid for biomarker analysis is challenging since a drop of sweat only contains a few microliters. But probably the main limitation of sweat-based analysis devices is the ability of sweat to dry quickly in air, which makes it unfeasibility for collection and use on chip for analytical purposes. The use of sweat for cancer detection and/or treatment monitoring has emerged slowly as a controlling tool, in amalgamation with SERS and other analytical detection techniques [71]. Recently, an assay combining high-resolution continuous source absorption spectrometry (HR-CSAS) and SERS has been developed to monitor fluorine-containing drug levels as a biomarker in sweat samples from cancer patients. The assay showed high sensitivity (up to 0.1 ng) at low sample consumption (30 nL). The method was fast, and no sample pretreatment was required to trace the fluorine signal. Importantly, the grouping of HR-CSAS with SERS helps in sample analysis before the sweat sample gets dried.

Proteins, such as pepsin (PS), and hemoglobin (HG), can serve as bioanalyte models in sweat. In order to aid in the breakdown of proteins and promote easier food digestion, the stomach produces the digestive enzyme PS. Meanwhile, the red blood cell's HG protein is in charge of transferring carbon dioxide from the body's tissues back to the lungs for expulsion and oxygen from the lungs to the body's tissues. However, for protein detection in SERS is challenging due to technique sensitivety,

Table 1
Advantages and disadvantages of the non-invasive biomarkers.

Advantage	Disadvantage
Fast	Not always sensitive enough
Serial collection	Highly sensitive devices have a high cost.
Easy replication	May require a high quantity of samples.
Cost-effective	
Simple procurement	

Table 2
Non-Invasive samples and SERS based detection for Cancer Biomarkers.

Sample type	Cancer Marker	Detected Cancer	Nanoparticle/ Assay	Reference
Sweat	Fluorouracil drug	Colon cancer monitoring	Ag colloids	[74]
Saliva	Proteins; amino acids; nucleic acids, thiocyanate, and phospholipids	Oral cancer	Au nanospheres	[78]
	Proteins and nucleic acids	Lung cancer	Ag colloids	[77]
	Alpha-fetoprotein	Hepatocellular carcinoma, breast cancer, and lung cancer	Au and Au@Ag Nanorods	[49]
Sputum	Proteins	Lung cancer	Au nanospheres	[64]
Urine	miRNA	Prostate cancer	Au nanospheres	[7]
	miRNA	Prostate cancer	Ag nanospheres	[48]
	RNA, microRNA	Prostate cancer	Au nanospheres	[97]
	Metabolites, proteins, and nucleic acids	Colorectal cancer	Au nanospheres	[98]
	Metabolites	Bladder cancer	Ag colloids	[99]
VOC	Aldehydes	Lung cancer	$CuFeSe_2$@Au nanospheres	[100]
	Aldehydes	Lung cancer	Ag nanospheres (superparticles)	[65]

and needs specific antibodies. Arabi and colaborates (2021) developed a capillary sensor with molecularly imprinted polymers (MIPs), act as synthetic receptors with molecular recognition sites akin to antibodies, can be produced through templating techniques within synthetic polymer networks. The response in sensing involves a shift in SERS intensity, and its interaction with Au through imprinted cavities designed within MIP. This platform presents a simple, and swift approach for the detection of biomacromolecules, such as proteins, demonstrating significant utility for quick and on-site practical bioassays at point-of-care settings [72]. A wearable and portable sensor was developed for monitoring urea and lactate levels in real-time and fresh sweat samples. This sensor is a plasmonic microfluidic patch that can analyze biomarkers using only 0.5uL of sample solution. Such capabilities are achieved through microfluidic techniques utilizing arrays of Ag nanomushrooms on Silicon wafers, enabling laser blockade against the skin [73]. The researchers were successful in gaining the molecular individualities of the metabolite of capecitabine by SERS demonstrating its potential applicability in therapeutic drug monitoring [74].

3.1.2. Cancer biomarker in saliva

Whole saliva is a readily available human fluid composed primarily of oral epithelial cells. Still, it also contains enzymes, such as amylase, for the preliminary digestion of food and mucosal healing, and proteins for mineralization of tooth enamel. This fluid presents several functions in the oral cavity, such as the protection of oral mucosa, oral homeostasis, or facilitation of taste perception. Importantly, saliva is considered a first line of defense against pathogens [75]. The amino acids and enzymes (Tyrosine and Coenzyme A, respectvelly) biomarkers are available in saliva may be altered in pathological conditions indicating the presence of nasopharyngeal carcinoma [76].

It has been reported that using silver nanoparticles, direct SERS analysis of a small amount of saliva was able to differentiate 21 lung cancer patients from 20 healthy subjects (with an 80 % accuracy) by measuring protein and nucleic acids [77]. In another work, oral cancer patients were distinguished from healthy subjects, and smokers from non-smokers through SERS analysis of thiocyanate levels in saliva samples [78]. The thiocyanate, a tobacco biomarker, shows a high level

Table 3
Invasive and non-invasive approaches for SERS based detection of Cancer biomarkers.

S NO.	CANCER MARKER	DETECTED CANCER	MARKER TYPE	NANOPARTICLE (NP) AND ASSAY	REF
1.	Erb2/EpCAM	Breast	Invasive	SERS tag (Au-rGO@anti-ErbB2), SERS CTC sandwich assay	[121]
2.	Label free cell DNA	Different kinds of tumor cells	Invasive	Direct SERS on Ag film substrate, DNA hybridization assay	[124]
3.	Keratin 18 EpCAM CD44 IGF-1	Breast	Invasive	SERS with Au@Ag nanorods, antibody assay	[166]
4.	EpCAM	Non-small-cell lung cancer	Non-invasive	SERS on Au NP + bifunctional molecule *p*-mercaptobenzoic acid (pMBA), Sandwich assay	[167]
5.	Her2 CD10	Breast, Lung	Invasive	silica-encapsulated magnetic NPs as SERS tags, SKBR3 antigen antibody assay	[168]
6.	Label free	Prostate cancer cervical carcinoma	Non-Invasive	Tailor-made membranes (MBSP) for Hela, PC3 cells, SERS using 40 nm of AuAg alloy	[169]
7.	PD-L1, MHC-I MHC-II, MCSP	Melanome	Non-Invasive	Anisotropic AuAg Alloy Nanoboxes codified with MBA, DTNB, TFMBA, and MMC as SERS tag	[170]
8.	ctDNA human blood	Cancer early diagnosis	Non-Invasive	SERS detection using single-walled carbon nanotubes with Cu NPs	[126]
9.	sDNA	Oral cancer	Non-Invasive	Ag NP and 4-mercaptobenzoic acid (4-MBA) as a Raman reporter, sandwich assay	[143]
10.	miRNAs	Breast cancer	Invasive and Non-Invasive	Various SERS tags Strategies	[132]
11.	α-fetoprotein Protein	Liver cancer	Invasive	Raman reporters microprinted on Ag NP films and conjugated with different ssDNA	[134]
12.	CD63	Human breast cancer cells	Invasive	Au@Ag nanorods codified with DTNB, exosome and HER2 antibody assay	[171]
13.	miRNA	Human breast cancer cells	Invasive	Au nanorods codified with QSY21 as SERS tag, Array of EpCAM, CD44, HER2, EGFR,	[149]
14.	MIF/GPC1/ CD63/EGFR	Pancreatic cancer	Invasive	Au@Ag NPs as SERS tags, sandwich assay for PATP-PDA-MIF/GPC1/ CD63/EGFR	[172]
15.	Plasma samples	Thyroid cancer	Non-Invasive	Reduced Ag NPs, Direct SERS assay on tissue sample	[173]
16.	Plasma samples	Bladder cancer	Non-Invasive	SERS with Ag NPs, Direct Tissue discrimination	[174]
17.	CD326/CD45	Breast cancer	Invasive	SERS on AgNPs direct cell detection	[175]
18.	AFP biomarker hepatocellular carcinoma	Liver cancer	Invasive	Label-free direct Raman detection	[176]
19.	NSCLC plasma	Lung Cancer	Non-Invasive	PCR-SERS, Ag NPs, EGFR genes, Aptamers assay	[177]
20.	Serum	Different stage cancer	Non-Invasive	Au NPs codified with 4-ABT as SERS tag, miRNA Assay	[178]
21.	Circulating miRNA	Lung Cancer	Non-Invasive	SERS with Au Nanotriangle, miRNA sandwich assay	[179]
22.	Whole blood	CTC cells	Non-Invasive	SERS with Ag NP codified with methylene blue for direct cell detection	[180]
23.	Rabbit blood	CTC cells	Non-Invasive	Au NP codified with 4-MBA as SERS tag, cell screening	[181]

in oral cancer samples, and thiocyanate's ions could also be detected in other chronic diseases such as emphysema or bronchitis, rendering broader options for applications of SERS [79]. Therefore saliva sampling can be a promising biofluid for cancer detection via SERS. We should point out that as diagnostic fluid saliva shows several advantages, apart from the richness in metabolites, such as non-invasive and convenient sampling and easy collection but also presents disadvantages as low concentration of some analytes, easy contamination (food, kissing, etc.), or abundance of proteins such as amylase, lysozyme, and mucin, which may interfere with the SERS analysis. Thus, for SERS analysis the saliva samples could need a pre-treatment step to remove high-abundance proteins. Researchers demonstrated SERS detection of saliva and serum samples for identifying Sjogren's syndrome (SjS), a general autoimmune disease, responsible for dehydration of the mouth and eyes. The samples were collected from a cohort of 29 patients suffering from SjS and 21 samples as control. SERS spectra were presenting the bands for purine metabolites such as uric acid, hypoxanthine, and xanthine. Primary module analysis-linear discriminant analysis (PMA-LDA) models were developed for statistical analysis of SERS spectra. An overall accuracy of 94 % for saliva and 98 % for serum was obtained which suggests that the SERS analysis can perform multiplex capture in complex biochemicals with Sjögren's syndrome biomarker [80]. In another study, researchers demonstrated a rapid mode for obtaining SERS signal of saliva samples. It implied mixing the saliva supernatant with Au nanoparticles and dropping it on a glass slide. A large area SERS mapping was acquired from both oral and healthy cancer samples. SERS signal presents fluctuations in intensity and frequency shifts with reproducibility. Thiocyanate, a biomarker present in saliva, was detected in the SERS spectra, for oral cancer sample types. The scrutiny showed that the cancer group exhibited an overall higher level at 2126 cm^{-1} band area, present C–N stretching vibrations of thiocyanate. The investigations confirm SERS technique can be effectively applied for non-invasive, label-free oral cancer detection [81].

3.1.3. Cancer biomarker in sputum

Sputum is a non-invasive sample that is more commonly used for detecting bacteria or viral infections [82]. Sputum, or mucus, has a functional role in humifying the airway, acting as a barrier [83]. This fluid is made of water (98 %), globular proteins (0.8 %), salt (0.9 %), and higher molecular weight polymers (0.3 %) in healthy person. The globular proteins are mainly composed of mucins, a large glycoprotein comprising regions with high concentrations of serine and threonine residues [84]. Currently, sputum has become a promising diagnosis fluid for the early-stage discovery of cancer, such as lung or oral cancer. Focused on SERS, Saranya and co-workers reported a study that developed SERS tags by codifying fluorogenic Raman reporters onto Au nanoparticles through a tripeptide for indirect multiplexed detection of lung cancer biomarkers (Fig. 3A) [64]. Besides, each of the SERS tags was conjugated with a monoclonal antibody Rh110-cathB@anti-CK, Coum-cathB@anti-Nap, and RhB-cathB@anti-EGFR are specific toward napsin-A (Nap), cytokeratin-19 (CK) and epidermal growth factor receptor (EGFR), respectively, which are specific biomarker commonly present in sputum sample of cancer patient [85] Researchers used SERS detection for tumor antigens which produce associated autoantibodies as prospective cancer biomarkers. A group of lung cancer (30 people) patients and 30 cancer-free smoking people were employed for the study. A panel of 3 antigen antibodies was developed in this research and achieved a sensitivity of 81 % and specificity of 83 % for the detection of lung cancer, despite their locations, stages, and types of lung tumors. The study proves it self a pioneer verification that sputum antigen antibody could be applied as biomarkers for the early-stage recognition of lung cancer [86]. Even though sputum is considered a non-invasive sampling method, it has important limitations that should be considered. Not all patients are able to release sputum samples for analysis, and even after the nebulization procedure, sample collection can still be a challenge. Sputum can contain normal cells and cancer cells in varying proportions, which can affect the accuracy of the diagnosis. Additionally, respiratory diseases can also affect sputum production, and for that the analysis of this biofluid.

3.1.4. Cancer biomarker in urine

Urine is a non-invasive body fluid rich in metabolites capable of reflecting the health conditions of an individual. The commonly available cancer biomarker are circulating free acids nucleic, and proteins. Cellular metabolism produces several by-products, such as creatinine, uric acid, or urea, which are concentrated with nitrogen and must be removed from the bloodstream [64]. Due to the simple collection of samples, urine is used to detect several diseases by analysis and characterization in patients of any group age (e.g., infants, children, seniors, pregnant women, and adults) [87]. As with other non-invasive samples, trained professionals are not essential for urine collection, and sample collection can be conducted at home by the patients themselves. Nonetheless, there are several challenges associated with obtaining urine biomarkers, such as: i) Sample variability: the composition of urine may vary depending on the time of day and individual's hydration status; ii) Contamination: urine samples can be contaminated by bacteria and other organisms, which may interfere with the analysis of biomarkers; iii) Sensitivity and specificity of biomarkers: some molecules may be present in low concentrations in urine depending on the detection method employed; iv) Material collection: standardized protocols for biofluid collection since external factors can alter urine composition and to interfere on results. Regarding SERS analysis several works have been reported. For instance, researchers demonstrated the detection of Prostate Specific Antigen (PSA), as biomarker for the diagnosis of prostate cancer (PCa), in urine samples (Fig. 3B) employing SERS tags based on Ag nanoparticles [66]. The PCa risk score was developed from the SERS intensity generated by the biomarker based on the Mi-Prostate Score (MiPS) algorithm. MiPS is a recently developed

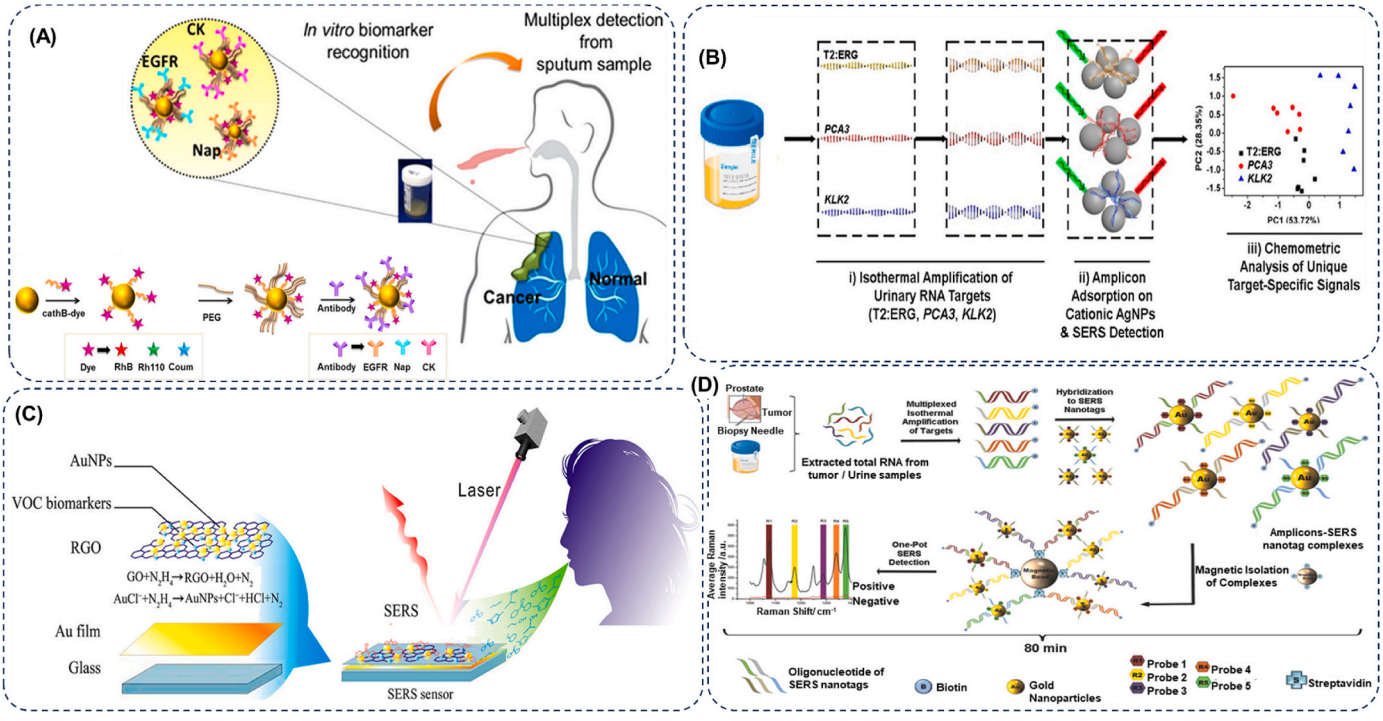


Fig. 3. **(A)** An example of sputum sample biomarker-based SERS detection for lung cancer patients [64]. *Copyright © 2018, American Chemical Society* **(B)** Silver nanoparticles applied to generate SERS signals for biomarkers to detect prostate cancer in urine samples [96]. *Copyright © 2018, American Chemical Society* **(C)** SERS sensor integrating reduced graphene oxide and gold nanoparticles to differentiate early gastric cancer [95]. *Copyright © 2016, American Chemical Society.* **(D)** Schematic representation invasive and non-invasive sample assay used with RNA biomarker extraction and amplification. Various SERS nanotags are used with complementary DN probe hybridization and purification steps. The multiplexed detection by SERS for quantitative analysis of biomarker is performed to achieve sensitive detection of each SERS nanotag in a very short time frame (80 min) of PoC application [60]. *Copyright © 2016 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.*

PCa risk-scoring test that aims to overcome the well-known shortcomings of the PSA test in the early detection of prostate cancer. This method was compared with the gold standard tissue biopsy results, and it was able to distinguish cancer patients from high-risk individuals with high accuracy, achieving 87 % sensitivity and 90 % specificity [66,88]. Moreover, it is demonstrated that patients with PCa exhibit elevated levels of miR-107 in their urine, which makes this molecule a promising biomarker for detecting prostate cancer. Based on this finding, Trau and co-workers developed a strategy for early PCa diagnosis based on the indirect SERS detection and quantification of miR-107. Thus, the existence of miR-107 triggers the self-assembly of Au nanospheres onto tunnelled Au/Ag alloy nanocuboids giving rise to plasmonic nanostructures with specific nanozyme action and high Raman signal improvement capability. Those properties were employed to improve the miRNA to clinically relevant levels without relying on PCR-based target amplification via nanozyme catalytic SERS signaling cascades. Since miR-107 target concentration could be correlated to Au nanozyme amount, the quantitative detection (till fM level) of miR-107 was achieved via the SERS analysis of 3,3′,5,5′-tetramethylbenzidine (TMB) oxidation products obtained under nanozyme catalysis of TMB in the presence of H_2O_2. Recently, SERS was combined for the first time with isothermal reverse transcription-recombinase polymerase amplification (RT-RPA) for subtyping prostate cancer from urine samples via multiplexed detection of RNA biomarker, as presented in Fig. 3D [7]. This strategy demonstrated an outstanding sensitivity of 200 zmol (100 copies) and specificity, which gives confidence in using it as a tool for rapid multiplexed subtyping of cancer. Besides prostate cancer, other cancer types have been diagnosed with high accuracy in urine via SERS, such as lung cancer [89], breast cancer [90], and colorectal cancer [91]. In breast cancer, a study has shown that distinctive SERS peaks of creatinine in urine samples. Additionally, they evaluated the relative efficacy of serum and urine SERS samples in the classification of control and breast cancer samples using five different machine learning algorithms. Similar overall performances ranging between 61 and 89 % suggest that both serum and urine hold promise as candidates for SERS-based liquid biopsy in breast cancer detection [92].

3.1.5. Cancer biomarker in Volatile Organic Compounds (VOCs)

VOCs are emerging biomarkers that can be collected from individuals at any location and are present in bodily fluids such as breath, blood, urine, and sweat [65]. The aldehydes exhaled in the breath can change during a pathological disease process, indicating oxidative stress and may be linked to the composition and metabolism of specific tumor tissues. Some aldehydes have already been identified as indicators of lung cancer [93]. The commonly available cancer biomarker are Polycyclic Organic Compounds (POCs), may be present in VOCs and are associated with certain types of cancer, such as skin cancer, and Polycyclic Aromatic Hydrocarbons (PAHs), that are recognized as carcinogens and can be detected in VOCs, primarily associated with lung cancer. The field of VOCs-based SERS assays is still emerging, where there are some limitations to be addressed such as weak Raman signals or high mobility of gaseous molecules, which results in low adsorption on solid substrates [94]. Qiao et al. found that the amalgamation of metal-organic frameworks (MOFs) and gold super particles (GSPs) allowed the enhancement of the VOCs adsorption on the plasmonic sensing platform [65] They employed a MOF made of Zn^{2+} ions and a 2-methylimidazole ligand, also known as ZIF-8, to coat the SERS-active GSPs. This porous material decreased the gas flow rate and directed the exhaled aldehydes to the GSP substrate via a ZIF-8 channel. Once there, the aldehydes react through a Schiff base reaction with Raman active molecules, 4-aminothiophenol grafted onto the gold surface, allowing the indirect SERS recognition of aldehydes with a limit of detection (LOD) of 10 ppb. Thus, the combination of MOFs with plasmonic SERS substrates has made the use of VOCs a promising approach for lung cancer screening. In another study, Cui and co-workers established a SERS sensor founded on the integration with reduced graphene oxide (RGO) and gold nanoparticles that allow to differentiate early gastric cancer from advanced gastric cancer (Fig. 3C) [95]. The role of RGO is the selective adsorption of VOC biomarkers avoiding interference from other gas molecules (H_2O, CO_2, or N_2). They demonstrated the detection of fourteen VOCs, such as acetone, isoprene, hexane, and menthol, among others in both real and computer-simulated breath samples. With the help of VOC biomarkers and detection methodology, it can detect gastric cancer and early gastric cancer in advanced gastric cancer patients in a rapid and cost-effective setting. Finally, the screening of these VOCs on 200 subjects demonstrated that this strategy allows accurate classification of early from late-stage gastric cancer patients with a sensitivity of 83 %, and a specificity higher than 92 %. However, more studies are necessary for applying VOCs in cancer screening programs especially thoracic and oral malignancies, such as lung and oral cancers.

3.2. Biomarker in invasive sample

The invasive type of cancer sample is usually obtained from the blood or the tissue sample from the operated part of the tumor. These tumor parts are usually obtained by an invasive surgical method. The blood test for cancer biomarkers detection is usually referred to as liquid biopsy and it can assist in screening, diagnosis, and therapy of cancer. The biomarker analytes employed in liquid biopsies can be divided into four major categories: CTCs, circulating nucleic acids, proteins, and circulating tumor vesicles (CTVs), which consist of exosomes and larger vesicles. In tissue samples, the most commonly used techniques are imaging analysis and chemical changes in biomolecules [101,102] the most commonly used technique to detect specific tissue protein biomarkers.

- **Circulating tumor cells (CTCs)**

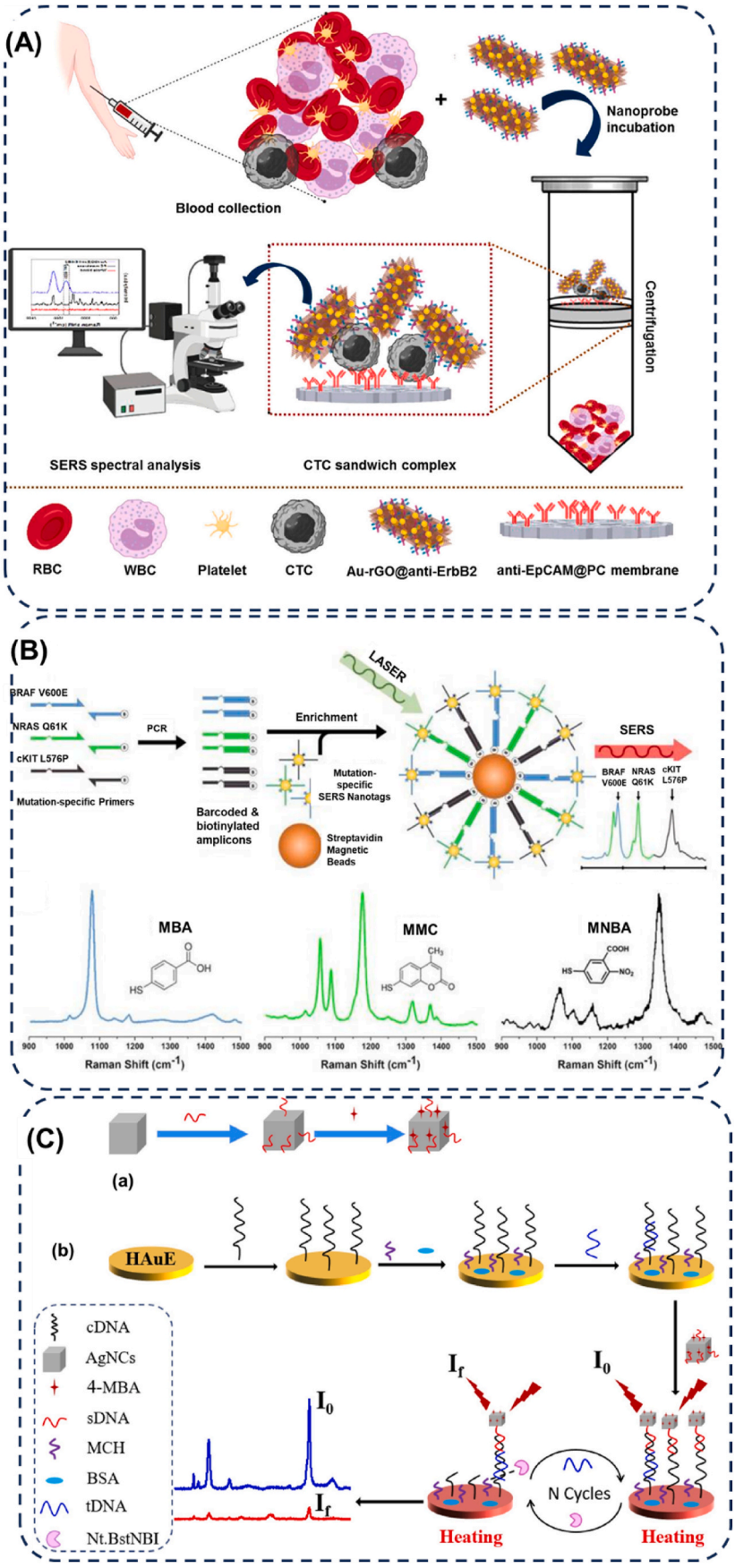
CTCs are epithelial cancer cells that can metastasize from tumors, spreading through the bloodstream and establishing secondary tumors at distant locations [103]. Therefore it is crucial in the process of tumor metastasis. The content of CTCs in peripheral blood is extremely low, as they are almost non-existent in healthy individuals, so their detection requires a very sensitive methodology. Since multiple studies have demonstrated a connection between CTCs levels and the survival of patients, CTCs are valuable biomarkers for early-stage cancer detection, prognosis, etc. For this reason, significant efforts are being made to develop a technology to detect and isolate CTCs. Many detection technologies of CTCs have been developed recently: fluorescence [104], colorimetric assay [105], electrochemistry [106], mass spectrometry [107], differential pulse voltammetry [108], or real-time PCR for gene expression analysis. All these methodologies can be implemented; however, they must meet stringent criteria in terms of cost, ease of operation, and required instrumentation [109]. Due to the low abundance of CTCs, reliable detection poses higher technical requirements for the more sensitive biosensors. SERS finds extensive application in the field of biology, chemical or environmental analysis [12,110,111]. SERS for the detection of CTCs has recently garnered significant attention owing to the inherent benefits of highly sensitive detection offered by SERS [112]. In 2008, Sha et al. [113] showed for the first time the capture and recognition of circulating breast cancer cells (cell line SKBR3) by combining epithelial cell adhesion molecule (EpCAM) conjugated magnetic beads and HER2 conjugated SERS tags. Thus, the magnetic beads and the SERS tags were incubated with SKBR3 cells spiked into whole blood (100 μL) for 30 min. Then this mixture was concentrated on the side of the tube using a magnet and the SERS analysis was performed. Thus, the LOD was 50 tumor cells/mL. Later this approach was validated with clinical blood samples and diagnosed with squamous cell carcinoma of the head and neck (SCCHN) [114]. In this work, CTCs concentrations of 5 cells/mL were detected without requiring any additional sample treatment. Interestingly, SERS spectroscopy may not necessitate the enrichment step typically required by

most CTCs detection methods [115]. In 2016, We et al. successfully demonstrated the detection of CTCs up to a LOD of 1 cell/mL without the need for any enrichment process using SERS tags [116]. Scarcity is not the only problem when quantifying CTCs. For example, the use of a single CTCs marker, typically EpCAM, can result in false positive or false negative outcomes [117]. Therefore, a specific panel of highly specific markers should be used to accurately differentiate CTCs. Recently, numerous research groups have demonstrated the multiplex detection of CTCs from other cells with remarkable precision via SERS. For instance, Cho et al. [118] employing up to five distinct recognition ligands to differentiate CTCs from other cells using SERS tags labeled with four different Raman reporters and each of them conjugated with a specific antibody for breast cancer marker (EpCAM, CD44, keratin, and insulin-like growth factor). The cancer stem cells (CCSC)-chip demonstrated detection efficiencies of 82.8 %, 91.8 %, 86.7 %, and 93.7 % for the detection of RAN (raman active nanoprobes)-labeled CCSC (cancer stem cells)/CTCs in the case of CCSC, MCF-7, MDA-MB-231, and SK-BR-3, respectively. In other work, researchers also demonstrated the detection of individual breast cancer cells in untreated human blood samples [119] this method enables the highly specific detection of a single cancer cell within a background of 7 million blood cells, without the need for enrichment, separation, or time-consuming procedures employed by alternative methods. This rapid and precise identification approach holds significant promise for early CTCs detection and timely initiation of cancer treatment. Also demonstrated the detection of individual breast cancer cells in untreated human blood samples [119]. . SERS tags were also employed for quantitative SERS analysis of MCF-7 type CTCs in a blood-mimicking fluid. The strategy allowed the rapid detection of 20 CTCs among many white blood cells. This study also demonstrated high selectivity since no SERS signal was observed when introducing HELA and HEK cancer cells into the blood-mimicking fluid. To establish a reliable and user-friendly platform for isolating CTCs that can be readily applied in clinical environments, Jibin et al. [120] developed a customized portable sensor platform. They utilized nanotag-enabled SERS filters to enable the specific targeting and isolation approach for CTCs of breast cancer cells (SKBR3 cells) in peripheral blood samples. The device employs centrifugal force based on size for the separation and isolation process, facilitating the efficient and accurate detection of CTCs. For this purpose, a strong and simple CTCs isolation platform was developed. The designed portable system, incorporating SERS nanotags, operates as a "lab-on-a-filter" (as presented in Fig. 4a), capitalizing on the target specificity of CTCs and utilizing size-based centrifugal force to separate and isolate CTCs from peripheral blood samples [121]. Prior to utilization in the developed system, the collected blood is subjected to incubation with SERS nanotags (Au-rGO@anti-ErbB2). For the preparation of the SERS tags, polycarbonate (PC) sheets conjugated with anti-EpCAM antibodies are employed, along with an anti-ErbB2 antibody. The centrifugal prototype accommodates the PC membrane filter in a manner that enables easy detachment and mounting for subsequent SERS imaging analyses. This placement facilitates the careful isolation of CTCs on the top surface of the filter. The inclusion of the SERS nanotag (Au-rGO@anti-ErbB2) in the structure facilitated the precise recognition of CTCs, playing a pivotal role in their isolation and quantification amidst the vast number of healthy cells availability in the bloodstream. To distinguish the captured CTCs, they employed fluorescence immunostaining with an anticytokeratin antibody, identifying CTCs as positive for both Hoechst and cytokeratin (CK). The mean recovery rates for 10, 25, and 50 spiked cells per 1 mL were 89.2 % ± 3.83 (n = 6), 84 ± 3.12 % (n = 6), and 83 ± 2.70 % (n = 6), respectively. When they used 5 cells spiked per 1 mL, the average recovery rate amounted to 56 ± 15.08 % (n = 6). This platform enables the rapid and direct isolation of viable CTCs from whole blood, eliminating the need for any prior sample pretreatment.

Another strategy for SERS detection of CTCs consists of using SERS tags conjugated with DNA strands that are specifically complementary to the mutated DNA sequences present in those cells. Through the assembly of gold nanoparticles in a triangular pyramidal DNA structure [122], created a nanosensor that provides more hot spots than typical nanoprobes and thereby improves the SERS signal. At a concentration of 5 cells/ml, they were able to identify epithelial cell adhesion molecules, which are present on the surface of CTCs but not blood cells. In certain instances, the utilization of label-free Raman spectroscopy for CTCs identification can circumvent the limitations associated with the methods. By collecting Raman spectra from cells, it becomes crucial to develop a suitable algorithm capable of swiftly and precisely distinguishing between the Raman spectra of blood cells and tumor cells to enable effective CTCs identification. In ideal circumstances, our method can detect HeLa cells within a range of 5–100,000 cells per milliliter (mL) when they are present in a background of 1.0 million cells per mL of HEK-293T cells. Furthermore, we observed a linear detection range of 3–500 CTCs per mL directly in human peripheral blood without the need for any enrichment process. In a study conducted by Fang and colleagues, the spectral properties of different types of tumor cells, white blood cells and red blood cells were examined using the silver film-based SERS technique. The researchers effectively identified the SERS spectra of these cells. They further developed a silver film SERS that integrated deep learning algorithms [123]. This technology demonstrated the capability to differentiate between blood cells and tumor cells rapidly and accurately, thereby providing valuable insights for label-free detection of circulating tumor cells [124].

- **Circulating Nucleic acid biomarkers**

Tumor cells in cancer patients' plasma have the ability to release DNA into the bloodstream, leading to higher DNA levels than in the plasma of healthy people. Circulating tumor DNA (ctDNA) shows promise as a biomarker because it forms a very small proportion (<1 %) of wild-type cell-free DNA and it can develop the recognition and observation of tumors in a non-invasive way [125]. ctRNA is another recognized category of circulating nucleic acids. In recent times, droplet digital PCR has been proven effective in precisely quantifying mutant copies from restricted DNA samples, such as ctDNA [126]. Novel approaches are still in demand to address the boundaries of present gold standards. Just a few works have reported ctDNA detection based on SERS and a real challenge is to develop fully portable ctDNA-based SERS devices [127]. Cao and co-workers describe how nanoparticles that are functionalized with oligonucleotides and Raman labels, combined with SERS, present a viable approach for conducting multiplexed discovery of oligonucleotide targets with the advantages of increasing the sensitivity and selectivity of detection [128]. Nanoparticles are being strongly advocated as the cutting-edge labeling technology for advancements in bio-diagnostic research. Interestingly, researchers developed, for the first time, a strategy for the ultrasensitive detection of broad-range ctDNA in human blood via DNA-mediated SERS using single-walled carbon nanotubes (SWNTs). Besides, a novel multiplex assay that combines PCR and SERS to detect different clinically important DNA anomalies in ctDNA for melanoma diagnosis (Fig. 4b) [129]. SERS technology was also combined with signal amplification assisted by nicking endonuclease and heated electrodes for achieving highly sensitive detection of DNA species associated with oral cancer [130]. Thus SERS tags based on silver nanocubes encoded with a Raman reporter and conjugated with signal DNA (sDNA) and heated gold electrode (HAuE) modified with a capture DNA (cDNA) hybridize the target DNA (tDNA) through the ends forming a sandwiched structure giving rise an intense SERS signal. In a further step, the detection of tDNA is amplified till 3.1 fM through an Nt. The process of target recycling amplification was facilitated by BstNBI by elevating the HAuE temperature to 45 °C (Fig. 4c). Initially, single-strand DNA probes were immobilized on the external surface of AgNCs through an Ag–S bond. Subsequently, SERS tags consisting of AgNCs/sDNA/4-MBA were generated after the assembly of 4-MBA onto AgNCs/sDNA the cDNA was attached to HAuE through sulfhydryl groups, followed by sequential modification with



(caption on next page)

Fig. 4. (A) A size-based centrifugal force separation for CTCs from peripheral blood samples [121]. *Copyright 2023 American Chemical Society*. **(B)** The scheme illustrates the multiplex PCR/SERS assay and the utilization of SERS nanotags. In this approach, specific primers targeting multiple mutations were employed to amplify tumor DNA. The resulting amplicons were then labeled with mutation-specific SERS nanotags, enriched through magnetic beads and finally detected by Raman spectroscopy. The spectral peaks indicate the presence of the mutation of interest [129]. *Copyright © Ivyspring International Publisher.* **(C)** The system presents the heating and hybridizing approach for detaching the SERS tag for signal determination [143]. *Copyright (2021), with permission from Elsevier.*

MCH and BSA to minimize non-specific adsorption. The immobilized cDNA on HAuE could form duplexes with tDNA, resulting in the formation of cDNA/tDNA duplexes. These duplexes contained the specific recognition sequence (5′-GAGTC-3′) for Nt. BstNBI. Subsequently, SERS tags were attached to the HAuE surface by hybridizing against the complementary end of the cDNA. This assembly of SERS tags generated the initial SERS signals (I0) on the sensing platform. Upon the introduction of Nt.BstNBI, the cDNA within the cDNA/tDNA duplexes underwent cleavage at a specific site located 4 bases away from the 3′ end of the recognition sequence. This cleavage resulted in the detachment of the cDNA segment connected to the SERS tags from the electrode surface. Consequently, the Raman signal associated with the detached cDNA was diminished. and the tDNA molecule was released from the cleaved cDNA/tDNA duplexes, making it available for the subsequent cycles of hybridization and digestion. The recycling mechanism allowed for the reuse of the target DNA, thereby achieving the purpose of signal amplification. Therefore, this platform can have enormous possiblity use for early clinical analysis of oral cancer.

Circulating RNAs (microRNA, and other non-coding RNA) have emerged as a novel biomarker for various aspects of cancer, including staging, diagnosis, prognosis, and monitoring therapeutic response. The reason is that ctRNA has been observed that extracellular nucleic acids are consistently released into the plasma of patients with diverse types of cancer [125]. DNA sequencing and PCR are the most classical methods to monitor ctDNAs in blood. In recent studies, it has been established that specific combinations of circulating miRNAs are dysregulated, indicating their potential as diagnostic markers and it has emerged as a novel disease biomarker such cancers and genetic disorders. There is strong evidence against miRNAs which provide evidence of working as oncogenes or tumor suppressor genes [131]. Two primary approaches have been utilized for the detection of miRNAs [132]. A DNA hydrogel-based multiplexed SERS sensor with great possibility for early screening and clinical analysis of cancer marked by miRNAs has been recently developed [133]. Zhu and co-workers suggested that combining the frequency shift in SERS detection in microcontact printing could be a viable option for a cost-effective and precise method to multiplex detection for serum microRNA. This approach shows potential for early detection and differentiation of primary liver cancers [134]. Zhou et al. [135] established a multiplex SERS-based sandwich hybridization approach for the recognition of various liver cancer miRNA biomarkers with Au nanoparticles as SERS tags (nanoprobes) and hollow silver microspheres Ag-HMSs as recognition substrates. Interestingly, the synthesized Au based SERS tags show controlled surface roughness, which significantly contributes to the high efficiency of the SERS tags. A mixture of multiple target miRNAs, previously trapped by capture DNA-conjugated Ag-HMSs, was recognized using SERS tags functionalized with different DNA probes (specific to hepatocellular carcinoma). The surface of Ag-HMSs was immobilized with capture DNA strands, and sandwich hybridization assays were conducted. The investigational findings demonstrate that the developed assay is specific with a LOD as low as 10 fM. The application of multiplex bioassays shows excessive potential for the discovery of various biomarkers, offering decent discrimination and sensitivity. It holds potential for multiplexed discovery of other biomarkers in diverse applications [135]. There is a need for further advancements in the progress of biochemistry and chemical SERS sensors, particularly those based on DNA/RNA, to facilitate the early identification of cancer-related diseases. Continued research and innovation in this field can lead to improved detection methods and enable early diagnosis of cancer and related conditions [136].

- **Protein biomarkers**

Early, rapid, and reliable cancer detection can provide a good prognosis and reduce mortality. It has been established that tumor development and occurrence are tightly correlated with tumor biomarkers. Conventional tumor biomarker detection methods, which rely on proteomics, metabolomics, and genomes, necessitate particular target markers and a significant investment of time and resources. A non-invasive, extremely sensitive, label-free vibrational spectroscopy method called SERS can identify biomedical alterations linked to cancer in biological fluids [137]. Xiong and coworkers [137], gathered a total of 110 serum samples, comprising 30 from healthy controls and 80 from cancer patients, including 30 with bladder cancer (BC), 30 with adrenal cancer (AC), and 20 with acute myeloid leukemia (AML). A minute amount of 1 μl of blood serum was combined with 1 μl of silver colloid and subsequently air-dried for SERS measurements. By utilizing spectral data augmentation, we implemented a one-dimensional convolutional neural network (1D-CNN) to distinguish between the three different types of cancer and healthy samples accurately and quickly, with an impressive accuracy of 98.27 %. Gradient-weighted class activation mapping, or Grad-CAM, was used to analyze the spectrum. This allowed the analysis to identify potential biomarkers by highlighting certain SERS peaks that corresponded to biological compounds. These included L-tyrosine in bladder cancer, acetoacetate and riboflavin in adrenal cancer, and phospholipids, amide-I, and α-Helix in acute myeloid leukemia. This discovery could provide important hints for comprehending the mechanism of intelligent cancer detection based on label-free serum SERS. The combination of deep learning and label-free SERS offers great promise for the rapid, accurate, and non-invasive detection of cancer, which could greatly improve precise clinical diagnosis. In other work Su and coworkers [138], introduce aptamer-peptide conjugates as an innovative type of SERS probe, designed for the direct and highly specific profiling of abnormal protein levels in cancer patients. These conjugates consist of an aptamer linked with glutathione (GSH), serving a dual role as the recognition element and the SERS reporters. Because of this design, SERS fingerprints for peptides and nucleic acids can be generated simultaneously. SERS frequency shifts-based immunoassays have proven to be remarkably suitable for the accurate identification of big macromolecules. In line with this approach, Zhao et al. [139], have engineered a SERS-based biosensor capable of simultaneously identifying both t-PSA (total) and f-PSA (free). This was accomplished by attaching the t-PSA antibody's carboxyl group to the 4-mercaptobenzoic acid (MBA) on the immunocapture substrate. The presence of t-PSA causes noticeable Raman frequency shifts in MBA. According to the results, t-PSA shows a dependable linear response in the concentration range of 1–200 ng/mL, but f-PSA exhibits a good linear response in the range of 0.1–20 ng/mL. In other study [140], used a customized microfluidic device intended for the detection of two gastric cancer (GC) protein biomarkers, carcinoembryonic antigen (CEA) and vascular endothelial growth factor (VEGF), to perform a SERS frequency shift experiment. The device has three parallel channel sets, each of which has two reaction areas. This design enables the simultaneous investigation of several biomarkers in different samples. The detection limit (LOD) achieved by the SERS microfluidic chip described is as low as 0.38 pg/mL for CEA and 0.82 pg/mL for VEGF. Soluble cancer protein biomarker detection, which is highly sensitive and multiplexed, has the potential to improve early cancer screening and offer real-time information on how patients are responding to treatment. However, the sensitivity of existing soluble cancer protein biomarker detection

technologies, including the enzyme-linked immunosorbent test, is limited. Additionally, these methods rely on costly monoclonal antibodies, which are subject to variations in quality [141]. Li et al., introduced a highly sensitive, cost-effective, and reliable surface-enhanced Raman scattering technology for the detection of a range of soluble cancer protein biomarkers. This panel includes soluble programmed death 1 (sPD-1), soluble programmed death-ligand 1 (sPD-L1), and soluble epidermal growth factor receptor (sEGFR), all of which play significant roles in disease progression and treatment effectiveness. The limits of detection of sPD-1, sPD-L1, and sEGFR were 6.17 pg/mL, 0.68 pg/mL, and 69.86 pg/mL, respectively.

SERS is a commonly employed technique for early cancer detection, known for its remarkable sensitivity and specificity. However, previous studies have been limited in scope, often focusing on a restricted selection of biomarkers, making them inadequate for detecting various types of cancer. Furthermore, these studies often lacked efficient data analysis methods. In contrast, the incorporation of machine learning techniques has demonstrated its worth in effectively classifying serum biomarkers to identify cancer and other diseases through omics data [54,55]. This research introduces SERS-AICS (Surface-Enhanced Raman Spectroscopy and Artificial Intelligence for Cancer Screening), an integrated method for liquid biopsy that uses silver nanowires to analyze serum samples. This method combines the processing of molecular vibrational signals with large-scale data mining algorithms. SERS-AICS not only successfully identifies samples at the early cancer stage, but it also successfully distinguishes pan-cancer patients from healthy controls with an impressive overall accuracy of 95.81 %, along with a sensitivity of 95.87 % at a specificity of 95.40 %, according to a study by Dong et al. that involved 382 healthy controls and 1582 patients from two independent cohorts. By resolving spectrum overlap concerns, SERS provides accurate chemical information that greatly improves multiplex detection's selectivity, specificity, and accuracy. Because of this, SERS-nanoprobe is a trustworthy molecular diagnostics tool. The ability to accurately estimate target biomolecules by label-free detection is a crucial expansion of SERS-based diagnostics, since many physiologically relevant compounds lack surface-seeking groups. However, label-based biomolecular detection using SERS provides a higher level of dependability. From certain biomolecules, it produces unique fingerprint signals that are critical parameters for both in vitro and in vivo investigations where high repeatability quantitative quantification is required. Advances in the cost-effective manufacture of substrates have led to the widespread use of SERS-based immunoassays in environmental monitoring, biochemical analysis, and clinical diagnostics. These applications have demonstrated comparable performance to the standard ELISA kits currently in use [142].

- **Extracellular vehicles**

Extracellular vehicles (EVs) are small membrane-bound particles that are available in various types of cells. They are responsible for cell-to-cell signaling and can carry a variety of biomolecules, including proteins, lipids, and nucleic acids, which can be transferred to recipient cells. EVs are classified based on their size, biogenesis, and cellular origin. The two main types of EVs are exosomes and micro-vesicles [144]. Exosomes, ranging from 40 to 150 nm, are small vesicles originating from the endosomal pathway, whereas microvesicles are larger vesicles (100–1000 nm) formed through the outward budding of the plasma membrane. EVs have been found to play important roles in cancer generation. In general, individuals with advanced cancer tend to exhibit a higher quantity of EVs compared to healthy individuals [145]. Thus, research on EVs is a rapidly growing field, and there is still much to be learned about their biology. Exosomes and macrovesicles are emerging as promising candidates for cancer biomarkers in clinical diagnostics and treatment [146]. SERS is also a promising technique for the detection and characterization of EVs, as it allows for the analysis of the molecular content of EVs with great sensitivity and specificity. In recent years, different SERS platforms have been developed for the detection of exosomes. Wang et al. showed the feasibility of using a multiplex EV phenotype analyzer chip (EPAC) to monitor patient treatment responses by analyzing the phenotypic changes in plasma EVs (Fig. 5b) [147]. EPAC integrates a nanomixing-enhanced microchip and a multiplex SERS nanotag system, enabling direct EV phenotyping without the need for enrichment (more details in Fig. 5b). In another study, a precision and efficient methodology was developed to simultaneously analyze multiple protein biomarkers expressed on small extracellular vesicles (EVs) derived from pancreatic cancer. This approach utilizes SERS tags and allows for the finding of multiple biomarkers in a single test, eliminating the need for complicated isolation procedures [148]. The quick phenotypic profiling of small EVs was achieved via analysis with a portable Raman of sandwich-type immunocomplex resulting after incubating SERS nanotags conjugated with antibodies against three specific EV surface receptors (Glypican-1, EpCAMs, and CD44 variant isoform 6 (CD44V6) and antibody conjugated magnetic beads in EV-suspended medium (conditioned EVs). The SERS assay showed a LOD of 2.3 10^6 EV/mL. By examining EV samples before, during, and following BRAF inhibitor therapy, the changes in phenotypic characteristics. This enables the assessment of treatment responses and the early detection of potential drug resistance. The study also demonstrated the different EV phenotypic profiles of small EVs from bladder, colorectal, and pancreatic cancer cell lines. Interestingly, Kiwizera et al. developed a cost-effective, portable, and efficient Raman-based exosome assay offering a straightforward and reliable method for exosome analysis. It consisted of an antibody-based capture array deposited on Au nanorod substrates for the detection and protein profiling of exosomes [149]. Consequently, the scientists were able to identify biomarkers for HER2 and EpCAM on exosomes found in the plasma of patients with HER2-positive breast cancer. This discovery emphasizes these macroporous biomarkers' potential for diagnosis. However, this method requires initial exosome isolation. Recently, by the combination of liquid biopsy with artificial intelligence and SERS, the simultaneous diagnosis of six types of early-stage cancers by direct SERS analysis of plasma exosomes was achieved [150]. Thus the acquired SERS signals of isolated exosomes are then analyzed with deep learning models. Similarly, Parlatan et al. ascribed EVs derived from a mixture of five different cell lines to their cellular origins using a machine learning-assisted SERS method [151]. The utilization of artificial intelligence in combination with SERS techniques allows for a novel approach to the label-free analysis of EV preparations. This method enables the differentiation between exosomes derived from cancer cells and those derived from healthy cells. Dong and co-workers [152], created a novel structure called an Au-coated TiO_2 macroporous inverse opal (MIO), taking cues from beehives. With the help of this structure's "slow light effect," SERS performance was significantly improved. Exosomes from cancer patients' plasma can be collected and analyzed using the MIO structure without the requirement for labeling steps. It was found that the P–O bond found in phosphoproteins and the SERS intensity at 1087 cm^{-1} work well together as a marker for liquid biopsies used in cancer diagnoses. It is noteworthy that exosomes retrieved from the plasma of cancer patients including those with prostate, lung, liver, and colon cancers have at least twice as much intensity of the 1087 cm^{-1} SERS peak as those from healthy people. This emphasizes how flexible and easy this method of diagnosing cancer may be.

- **Imaging analysis and chemometrics of biomarkers.**

Tissue biomarkers are gaining prominence in scientific research, disease diagnosis, and predicting treatment outcomes. The use of quantitative image analysis has become an essential tool for the comprehensive exploration of tissue biomarkers in these specific domains [102].

Tissue samples are commonly used in cancer diagnosis, staging, and treatment planning. To obtain a tissue sample for cancer analysis, a

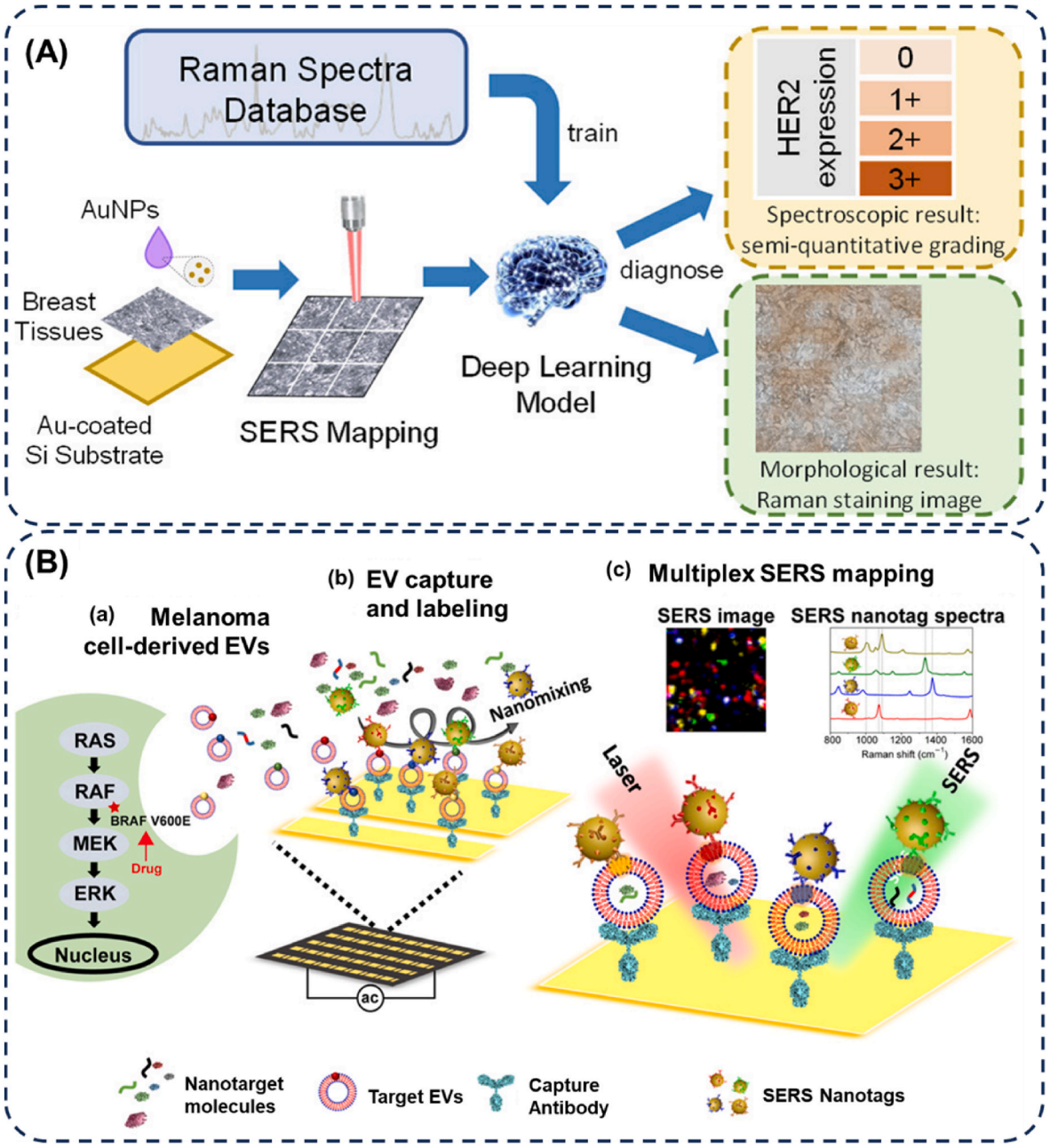


Fig. 5. **(A)** Schematic of immunohistochemistry analysis of HER2 combined spectroscopic and morphological diagnostic method based on label-free surface-enhanced Raman scattering (SERS) [163]. *Copyright 2023 American Chemical Society*. **(B)** Schematic for EV phenotyping by phenotype analyzer chip. (a) A melanoma cell harboring a BRAF V600E mutation releases extracellular vehicles (EVs) into the bloodstream or cell culture medium. (b) The sample containing these EVs is directly introduced into the EPAC (EV Phenotype Analyzer Chip), where a nano mixing technique is employed to enhance interactions between the EVs, capture antibodies, and SERS nanotags. This process selectively removes non-target molecules and unbound SERS nanotags. (c) The EV phenotypes are then characterized using SERS mapping, where false-color SERS spectral images are generated based on the intensity of characteristic peaks exhibited by the SERS nanotags [147]. *Copyright 2021 American Association for the Advancement of Science.*

biopsy or surgery is performed to remove a small amount of tissue from the suspected cancer site. Tissue samples are also used to monitor the effectiveness of cancer treatment over time. Overall, tissue samples are an essential tool in cancer diagnosis and treatment, and they provide critical information that can help guide personalized treatment decisions [4]. SERS has shown promise as a promising tool for the recognition and analysis of cancer in tissue samples. SERS can enhance the Raman scattering signal from molecules within the tissue sample, allowing their detection with high sensitivity and specificity [153–155]. In cancer tissue analysis, SERS can detect biomolecules associated with cancer, such as nucleic acids, proteins, and lipids, or even differentiate between cancerous and non-cancerous tissue samples [156]. Zhang et al. [157] present a novel stimulus-responsive SERS (SR-SERS) probe designed to act as a "Trojan horse" in the tumor microenvironment (TME) to enhance vibrational signals, enabling precise molecular diagnosis of cancer. This probe demonstrates responsiveness to specific stimuli, allowing targeted amplification of SERS signals within the TME. A TME-specific SERS probe has been developed, utilizing gold nanoparticles encapsulated with manganese dioxide (Au@MnO_2 NP). This innovative SR-SERS probe was utilized to accurately identify the tumor-to-normal tissue margin. Moreover, the SR-SERS technique was employed to differentiate molecular fingerprints of tumors at different

growth stages, providing valuable insights into tumor progression.

Another study demonstrated the feasibility of using SERS tags for quantitative multiplexed molecular imaging of freshly excised human tissues, with the aim of providing surgical guidance applications [158]. The SERS imaging of tumor sample of xenografts and human breast tissues presents the potential of using non-targeted SERS nanoparticles for quick (<15 min) phenotyping of tissues. This approach could facilitate the intraoperative recognition of carcinoma at lumpectomy margins. Girish and colleagues offered a concept for a SERS device designed for the detection and classification of oral tissue Raman signatures. The device demonstrated the ability to differentiate between healthy, premalignant, and malignant samples, providing potential applications in oral tissue characterization. The sensor element of the catheter consists of a SERS substrate composed of sheet-like TiO_2 nanostructures (30 nm) Ag nanoparticles. The device undergoes testing using 37 patient samples, including malignant oral squamous cell carcinoma (OSCC), premalignant leukoplakia, verrucous carcinoma, and disease-free conditions. It achieves an impressive precision of 97.24 % in detecting and classifying these oral tissue conditions, all within a time of approximately 25–30 min per patient [159]. Thus, SERS was used to analyze tissue samples from patients to classify bladder tissue as normal or cancerous [160]. This study addresses the challenge of bladder cancer by evolving targeted intravesical nanoparticles based on SERS technology. This method was inspired by the possible advantages of intravesical SERS nanoparticles over other contrast agents. The goal of this study was to evaluate the advantages and contests linked with active and passive control of bladder cancer through the application of nanoparticles. The specific outcome of the work is finding proof for passive targeting of nanoparticles within the bladder, enhanced penetration and retention of nanoparticles when applied topically and use of multiplexed molecular Raman imaging to bladder tissue is classified as normal or neoplastic. The enhanced surface permeability observed in this study provides a straightforward targeting mechanism that we anticipate will assist in the diagnosis and treatment of bladder cancer. Li and colleagues [161], employed alkynyl and nitrile-functionalized molecules to precisely construct a series of SERS tags for multiplex imaging. Alkynes and nitrile groups are exactly bound to the surface of metal nanoparticles and generate robust Raman signals. They have established an archive of Raman reporters comprising functional groups and presented a method to formulate SERS tags for multiple detection of cancer biomarkers. These SERS tags were subsequently linked by distinct antibodies targeting epidermal growth factor receptor (EGFR), progesterone receptor (PR), and estrogen receptor (ER), enabling the visualization of distinct biological aims in human breast cancer tissues through multicolor imaging. In conclusion, SERS tags exhibited their capability to detect and analyze multiple biomarkers expressed in cancer cells and human breast cancer tissues. This confirms their immense potential for complex imaging in clinical analysis and holds promise for advancing diagnostic techniques in the field. Feng et al. showed a study to investigate the potential of utilizing silver nanoparticle-based near-infrared SERS spectroscopy in combination with PCA and linear discriminate analysis (LDA) for distinguishing esophageal cancer tissue from normal tissue. SERS technique was applied to analyze samples of esophageal tissue, including both normal tissue and cancerous. The findings of the study indicate the feasibility of employing silver nanoparticle-based SERS detection in combination with PCA and LDA. This approach demonstrates the potential to accurately separate esophageal cancer tissue from regular control tissue, achieving extreme analytical understanding and sensitivity [162].

Mo and coworkers developed a strategy for the recognition of HER2 expression in breast cancer tissues using a combined morphological and spectroscopic method via label-free SERS (Fig. 5a) [163]. The approach presented in the study enables not only quantitative detection of HER2 expression but also provides information on the spatial distribution of HER2 in breast tissue. The proposed approach relies on establishing a robust SERS spectra archive of HER2 and breast cancer tissues, followed by utilizing a model for sample analysis. This strategy successfully distinguishes between the Raman spectra of breast cancer and normal tissues associated with HER2. It offers highly sensitive quantitative recognition at the molecular level and in clinical explanation and management. Karunakaran et al. [164] developed a strategic method for differentiating cervical exfoliated cell samples into high-grade intraepithelial lesion (HSIL), cervical squamous cell carcinoma (CSCC), and normal (NRML) using label-free, highly sensitive SERS. They employed three different techniques: single-cell analysis, cell-pellet analysis, and extracted DNA analysis. Using a SERS substrate based on Au nanoparticles, characteristic Raman features related to carotenoid/glycogen and amide III/nucleobases were evidenced allowing the empirical differentiation of the cell classes. Additionally, chemometric methods, such as Support Vector Machine (SVM), produced average diagnostic accuracies for the three grades of 94 %, 74 %, and 92 %, respectively. In areas with limited resources, the combination of machine learning and SERS analysis holds promise for field trials and has showed the potential to reduce the prevalence of cervical cancer.

For clinical diagnosis, a novel histopathological method utilizing indirect SERS was reported [165]. This method uses SERS nanotags to enable the rapid, highly sensitive, and accurate multiplex detection of three important breast cancer biomarkers, ER (estrogen receptor), PR (progesterone receptor), and HER2, in a single tissue sample. Notably, a sensitivity of 88 % and a specificity of 85 % were achieved for the duplex biomarker, 75 % and 67 % for the triplex biomarker, and 95 % and 92 % for the singleplex. While SERS has shown promise for the analysis of cancer tissue samples and personalized treatment plans for cancer patients, further research is needed to fully understand its potential and limitations.

4. Portable SERS based diagnostic systems

Benchtop Raman systems are intended to be utilized in a laboratory setting, where they can be combined with sophisticated optical interfaces for sample movement and/or imaging, top-tier detectors, and various excitation wavelengths to optimize research versatility. With the help of complex software, they can automatically acquire and process signals. Their great size, weight, and cost make them unsuitable for use in field research. The user can streamline and design a Raman system to their unique application with portable Raman spectrometers, also known as “compact” Raman spectrometers, minimizing weight, size, cost, and complexity. They can be compact and sufficiently sturdy to be easily transported between locations if they are designed with an integrated excitation laser. They can also be strong enough to be used in numerous studies. They provide choices for excitation wavelength, level of integration, and sample interface. Software for gathering and analyzing data is included, and they are operated with a laptop or tablet. An excellent tool for field research and measurements, as well as for application specific. Early diagnosis and treatment give patients a chance of survival and significantly increase their chances of cure. The present gold standard biopsies are good to provide a timely diagnosis of CTCs from a blood sample. Most clinical and immunochemical tests are performed in sophisticated laboratories using high-throughput screening technologies. It involves commercially available standard technologies and portable devices which allows greater control and analysis of samples. A portable point of care (PoC) device reduces the need for big laboratories and provides timely and accurate detection of molecular biomarkers. Progress in PoC technology allowed various evaluations and testing on-site. Recently scientists have developed SERS based PoC assays for the direct revealing of cancer. These assays have the potential to be adopted for several cancer diagnostics that also include tissue biopsies. For instance, using neck cancer as model cancer and micro-RNA biomarkers for detection, a SERS based assay was tested with 20 clinical samples and demonstrated good accuracy in the recognition of squamous cell carcinoma (Fig. 6A). A portable Raman device has achieved a specificity of 97 % which presents the promises of

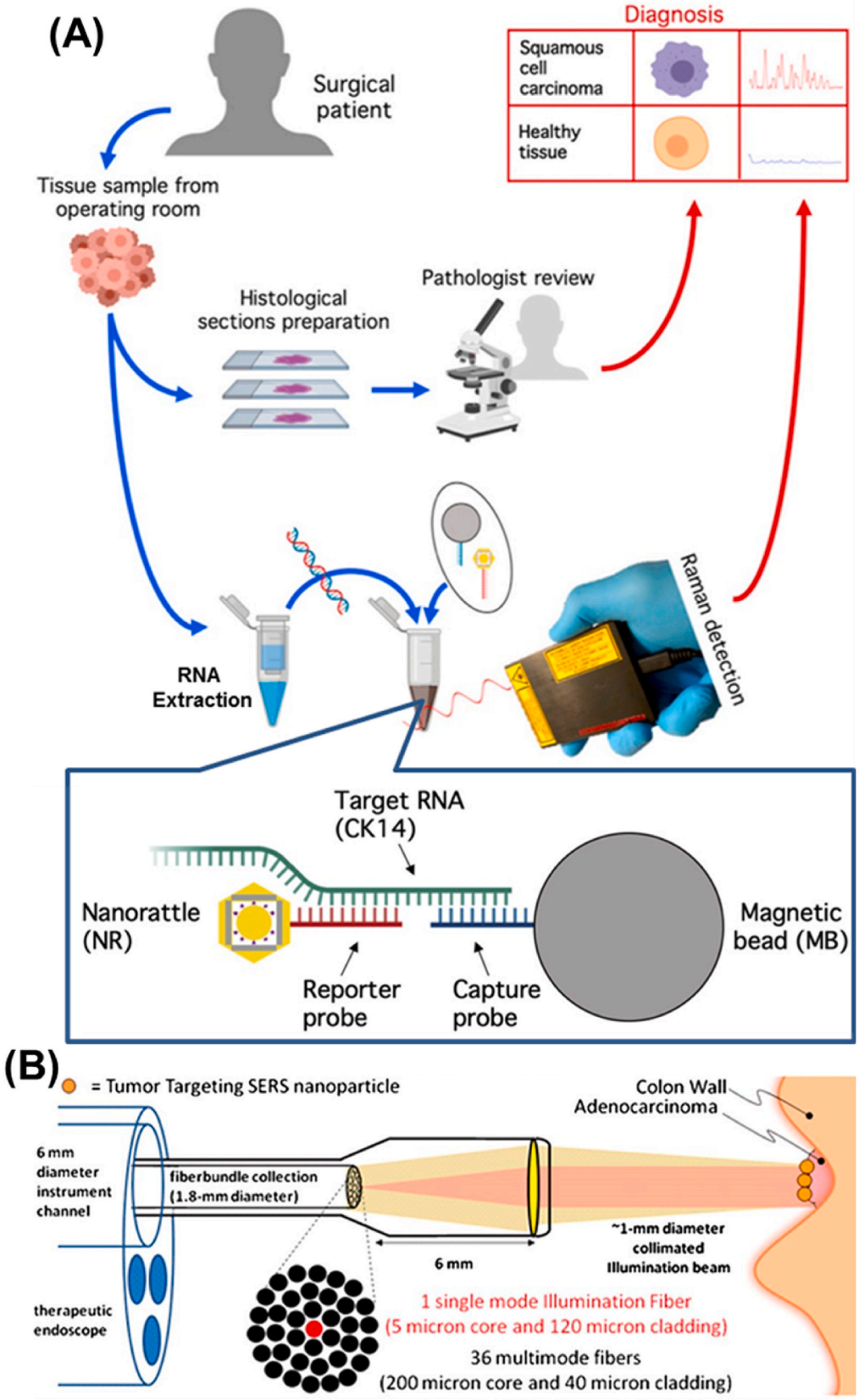


Fig. 6. **(A)** Illustration of a plasmonic based POC device for direct detection of cancer in clinical samples using a magnetic bead-based RNA sample extraction and purification system. Inset the hybridization mechanism and elements [182]. *Copyright © 2020 Published by Elsevier B.V.* **(B)** A portable Raman based endoscope developed to perform clinical procedures. It used a single-mode illumination fiber surrounded by multimode collection fibers. The excitation laser light illuminates a spot size of ~1.2 mm in diameter. This helps to perform direct detection at the molecular level [183]. *Copyright © 2013 Published by PNAS.*

POC diagnosis of cancer in low-resource settings [182]. A minimally invasive approach based on endoscopic imaging was developed using a unique fiber optic-based Raman spectroscopy device. This portable device provides real-time and multiplexed information and can be used as a clinical endoscope to identify and measure the presence of tumors by targeting SERS tags (Fig. 6B). A LOD of 326-fM (concentration of SERS tags) with a working distance from 1 to 10 mm was achieved [183]. Moreover, it allows endoscopists to recognize between normal and cancerous tissues promptly. The use of a highly sensitive SERS molecular imaging contrast agent enables the rapid and on-site diagnosis of cancer. This contrast agent exhibits excellent recognition capabilities and the ability to perform multiplexing, allowing for the real-time recognition of multiple cancer biomarkers. This technology provides real-time diagnostic information, aiding in the prompt and accurate diagnosis of cancer. A developed Raman endoscope can also be applied to detect spectral marks linked with targeted SERS tags. Shu and co-workers developed a portable SERS based proper screening and validation system for early-stage cancer prognostic [184]. The device works as a semi-automated powerless photoelectrochemical immunosensing platform. It consists of (1) a photoanode made of Au–$BiVO_4$ nanocomposite with Au nanocrystal on its high-active {010} facets to produce photocurrent signal, (2) magnetic beads conjugated with monoclonal anti-PSA capture antibody as the immunosensing probe and (3) Au nanoparticles conjugated with glucose oxidase/monoclonal detection antibody as the signaling probe. The system presents a LOD of 4.0 pg/mL.

5. Future prospects

The application of SERS spectroscopy to diverse biomarkers uses different strategies to achieve maximum sensitivity and multiplexing capability. The multiplexed, label-free/direct approach is attracting attention with machine learning automation and data analysis overcoming the limitations associated with the SERS tag approach. There is still a long way to go before we see this approach in clinical settings. By integrating plasmonic nanoparticles with controlled fluid flow, multiplexed platforms in conjunction with SERS technology have demonstrated the ability to detect biomarkers at trace concentrations with high sensitivity. These SERS-microfluidic platforms offer a promising approach to precision oncology, enabling rapid and accurate cancer screening. This technology has the potential to greatly enhance the efficiency and effectiveness of cancer screening processes, leading to improved patient outcomes and earlier detection of cancer. In the review, we tried to explore various methods and approaches in different types of invasive and non-invasive biomarker-based assay development and fabrication of SERS nano-biosensors for PoC diagnosis of various types of cancers.

The future direction of biomarker based PoC devices depends on automation, machine learning, and artificial intelligence. Automated technology in clinical data has increased swiftly for improving precision, efficiency, and monitoring at remote sites in the near-term forthcoming. Portable wireless devices are changing the way healthcare services work and ensuring reliable detection. The AI based software and devices can screen multiple biomarkers at the same time and enable the detection of interactions which is difficult to achieve in manual approaches. The important limitation in the progress of SERS based PoC systems is variability in substrate and methodologies used for the detection of biomolecules. The research conducted by previous scientists primarily revolved around studying the SERS spectra of important analytes and substrate materials. This emphasis has generated considerable interest in recent times to further enhance sensor capabilities, explore novel substrate materials, and improve sensitivity levels. Current efforts are directed towards advancing the overall performance of SERS sensors by developing innovative sensor technologies, investigating new substrate materials that exhibit improved SERS activity, and implementing strategies to enhance sensitivity and selectivity. These endeavors aim to push the boundaries of SERS-based sensing, enabling highly sensitive and accurate detection in various fields, including biomedical diagnostics, environmental monitoring, and chemical analysis. It is expected that in the coming time, this focus will be centered around the validation and multiplexing of the system. This is vital for such a tactic for clinical detection and will require resources and innovation in scaling up with accuracy and precision in the technology. Future technologists face challenges in meeting the needs of the medical industry. Substantial funding and commercial support would be a plus for progress in cancer diagnostics. Considering modern advances in biosensors, PoC systems must overcome some hurdles to be used for early clinical diagnosis of cancer. Firstly, we need to have innovative on-a-chip microfluidics systems, and secondly, improve sensitivity and specificity and reproducibility of PoC devices. Although several devices show good efficacy in the laboratory environment, in field conditions with real samples they lose their efficiency. It is also very critical for the selection of biomarkers to be analyzed in real samples. Third, cost-effective manufacturing is also important for affordability. For instance, the use of new technology such as 3D printing might help in prototyping and reduce production costs. PoC diagnostics also has the advantage of transportable, and on-site detection, which is important for the demand, for a fully functional PoC system to extend diagnostic opportunities.

CRediT authorship contribution statement

Lorena Vázquez-Iglesias: Writing – original draft, Writing – review & editing. **Giovanna Maria Stanfoca Casagrande:** Writing – original draft, Writing – review & editing. **Daniel García-Lojo:** Writing – original draft, Writing – review & editing. **Letícia Ferro Leal:** Writing – original draft, Writing – review & editing. **Tien Anh Ngo:** Writing – original draft, Writing – review & editing. **Jorge Pérez-Juste:** Writing – original draft, Writing – review & editing. **Rui Manuel Reis:** Writing – original draft, Writing – review & editing. **Krishna Kant:** Writing – original draft, Writing – review & editing. **Isabel Pastoriza-Santos:** Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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