Surface enhanced Raman spectroscopy (SERS) for *in vitro* diagnostic testing at the point of care

Haley Marks, Monika Schechinger, Javier Garza, Andrea Locke* and Gerard Coté

Abstract: Point-of-care (POC) device development is a growing field that aims to develop low-cost, rapid, sensitive *in-vitro* diagnostic testing platforms that are portable, self-contained, and can be used anywhere – from modern clinics to remote and low resource areas. In this review, surface enhanced Raman spectroscopy (SERS) is discussed as a solution to facilitating the translation of bioanalytical sensing to the POC. The potential for SERS to meet the widely accepted “ASSURED” (Affordable, Sensitive, Specific, User-friendly, Rapid, Equipment-free, and Deliverable) criterion provided by the World Health Organization is discussed based on recent advances in SERS *in vitro* assay development. As SERS provides attractive characteristics for multiplexed sensing at low concentration limits with a high degree of specificity, it holds great promise for enhancing current efforts in rapid diagnostic testing. In outlining the progression of SERS techniques over the past years combined with recent developments in smart nanomaterials, high-throughput microfluidics, and low-cost paper diagnostics, an extensive number of new possibilities show potential for translating SERS biosensors to the POC.

Keywords: optical biosensing; point of care; surface enhanced Raman spectroscopy.

1 Overview of point-of-care technologies

Patient care and diagnosis revolves around understanding an individual person’s unique and complex physiology and is historically done through the collection and analysis of bodily fluids. Blood, urine, and sweat (BUS) are the most common biological samples collected from patients and used in laboratory testing. However, various other bodily samples, such as cerebral spinal fluid or sputum, can also be used for testing but can be much more difficult to obtain from the patient or are less well studied in terms of their correlation with the known clinical blood biomarkers [1].

Results obtained from BUS assays provide physicians with valuable insights for improved diagnoses, monitoring, and treating of patients [2]. Therefore, it is understandable that over 13 billion *in vitro* diagnostic tests (IVDs) are performed annually [3]. To drive healthcare forward and facilitate more predictive, personalized medical care, the testing of biological fluid using point-of-care (POC) technologies will become increasingly paramount [4, 5].

In the US, 86% of all IVDs are performed off-site at independent laboratories or centralized hospitals [3]. Patients are typically referred to a separate location where biological samples can be collected and analyzed by a phlebotomist and trained technician, respectively. Laboratory testing of patients in underserved areas including developing countries presents even greater challenges due to the limited access to laboratory equipment, clean water, and consistent electricity. Patient samples are typically sent to off-site locations capable of performing such tests, which runs the risk of samples being contaminated, lost, or mislabeled [6]. Many patients in underserved settings are unable to return to city clinics for multiple appointments and often never receive results, only the initial medication, further prolonging proper diagnosis and treatment [7].

The hassle of off-site laboratory testing and the stress of waiting for results motivate the need and demonstrate opportunity for improvement and innovation in this area of healthcare. Recent developments in POC technology focus on creating rapid diagnostic tests (RDTs) where results are obtained minutes after collection, yet many of these tests are not as accurate as their laboratory-based equivalents. Enhancing these tests through the development of POC technologies that involve automated sample...
preparation, such as microfluidic lab-on-a-chip (LOC) devices have the potential to remove the need for complex laboratories and experienced personnel. If they can be made equally as accurate, robust, and easy to use this could improve overall health quality by bringing BUS test results to the patient’s bedside, into the home, inside the ambulance, or on the field [8].

2 Defining the ideal characteristics of a POC technology

A POC technology should ideally be accurate, portable, simple to use [2], and easy to read a positive or negative result; require little or no sample preparation; provide timely results; and be cost-effective [9]. The goal of POC technology is to provide a user-friendly interface, without the need of expert technician support or complex analysis, while meeting the current clinical chemistry standards for IVDs as set by the AACC [10, 11].

The World Health Organization (WHO) has defined a set of parameters that outline the specifications for POC technology in developing areas. This outline was originally created for the development of portable devices for human immunodeficiency virus (HIV) testing. The parameters are commonly referred to as the ASSURED criteria [12]. According to the WHO, POC technology must be affordable for patients without creating financial strain, sensitive to detect the biological target molecules at physiologic concentrations comparable to the central laboratory result, specific to only the target biomolecule and impervious to comparable molecules, user-friendly and easy to use with little or no training or expertise necessary, rapid and robust to provide timely results using samples that can be handled under the POC condition needed for the setting, equipment that is large and inconvenient should be avoided, and deliverable to patients in need at their location. These parameters have been well accepted and are the general standard criteria for classification as a POC technology [13].

2.1 Current commercial POC technologies

There are POC devices that are commercially available. The most familiar example is the glucose meter for managing diabetes, a chronic illness effecting 29 million people in the US alone [14, 15]. It is defined as a group of metabolic diseases where ultimately the body’s pancreas does not produce enough insulin or does not properly respond to insulin produced, resulting in high blood sugar levels over a prolonged period. Glucose meters and other POC devices utilize an assortment of methods for detecting and monitoring biomarkers including electrochemical [16–20], magnetic [21–30], optical [31–34], label-free spectroscopic analysis [35–43], colorimetric [44–49], and plasmonic nanoparticle based sensors [50–52]. Generally, electrochemical detection uses potentiometric, amperometric, and impedimetric measurements in conjunction with electroactive tags or free flowing electroactive analytes [17–20].

Many of the commercial glucose detection monitors One Touch Ultra (Johnson & Johnson, Wayne, PA, USA), Arkray (Edina, MN, USA), Ascensia Contour (Ascensia, Parsippany, NJ, USA), BD Test Strip (Becton Dickinson, Franklin Lakes, NJ, USA), FreeStyle and Precision Xtra (Abbott Laboratories, Chicago, IL, USA), TrueTrack Smart System (Trividia, Fort Lauderdale, FL, USA), Accu-chek Aviva (Roche, Basel, Switzerland), etc. [15, 53, 54] are examples of electrochemical and colorimetric devices that use test strips. In addition, the i-STAT monitor (Abbott Laboratories, Chicago, IL, USA) is an electrochemical approach that uses individual microfluidic cartridges for monitoring lactate, pH, \( \text{pO}_2 \), \( \text{pCO}_2 \), electrolytes, and hematology [55].

A newer commercial POC approach that uses magnetic nanoparticles, an antibody immunoassay, and optical detection to measure troponin, one biomarker for detection of myocardial infarction (heart attack), was recently developed by Philips (Handheld Minicare I-20). The system was created for use in emergency departments and provides results in 10 min from a droplet of blood instead of the typical 1-h wait incurred using central laboratories [56, 57].

Other examples include the mChip, a device that tests whole blood for HIV using a combined microfluidic and protein-based immunoassay approach [58], and the Ativa Micro Lab (Ativa Medical, St. Paul, MN, USA), which was recently shown to execute a number of distinct blood tests with the use of a single device [59–61]. One upcoming technology is Ativa’s portable Micro Lab, which has the potential to perform up to 25 distinct blood tests utilizing its various disposable test cards for each test. The Micro Lab system uses microfluidics, flow cytometry, electrochemistry, and colorimetric readouts in combination with established imaging techniques to conduct and relay important diagnostic information. While not yet approved by the FDA or commercially available and more of a bench-top reader rather than a hand-held POC device, it shows promise as a potential portable and user-friendly instrument based on a microfluidic cartridge suited to measure several tests.

One of the most popular emerging POC techniques that are commercially available for some applications are based on lateral flow assays (LFAs), which use colorimetric barcodes that can be viewed with the naked eye, absorbance measurements with a cell phone, or fluorescence techniques.
to quantify various biomarkers. The most popular LFA is the pregnancy test First Response (Church & Dwight Co., Inc., Trenton, NJ, USA), Clearblue (Swiss Precision Diagnostics GmbH, Geneva Switzerland) etc. [1, 4, 62]. The technology is based on porous paper that contains an immunoassay, gold nanoparticles, and a porous membrane capable of transporting fluid (e.g., urine). As the solution migrates down the paper, the target molecule, sometimes referred to as the antigen, chemically reacts with its antibody that has been immobilized on a particle surface. The particles can then be trapped downstream in the strip with another antibody and read with an optical reader for quantification or a yes/no readout can be obtained by visual inspection [63]. Although there are many POC devices commercially available, they are primarily designed to produce semi-quantitative or quantitative information for a single analyte per test [10]. Thus, the common limitations of commercially available POC devices are accuracy, variations of cost per test, and the inability to simultaneously monitor multiple analytes. All of these factors are important and often required to make definitive diagnoses [61, 64–67]. As a consequence, research and engineering efforts are still required to address these factors to improve POC technology.

2.2 Optical analyses for facilitating POC technologies

Current research efforts are directed at developing POC technologies with the necessary performance to be useful for diagnostics. In addition, physicians and clinicians are interested in the emergence of POC devices capable of simultaneously sensing multiple biomarkers and analytes characteristic of specific disease states [9, 68]. The additional push for one technology to monitor several biomarkers simultaneously for more effective and universal disease diagnosis is motivating recent advancements in multiplexed sensing capabilities.

Many optical approaches have been utilized for POC applications including fluorescence [69–72], luminescence, absorbance, Forster energy transfer (FRET) [73], bioluminescence energy transfer (BRET) [74–76], surface-plasmon resonance (SPR) [77, 78], resonant Rayleigh scattering [79–82], geometric scattering [1], and Raman spectroscopic approaches [83–89]. Advancements in biochemical sensing methods, nanotechnology, and the miniaturization of optics have been key in improving spectroscopic platforms for biomarker detection.

In many cases, optical approaches are more successful in simultaneously sensing multiple analytes through the use of photoactive dyes conjugated to biorecognition ligands [33]. Indirect sensing through the identification of optically active molecules, including chromophores and fluorophores or other reporter molecules, has improved the sensitivity and multiplexing capabilities of techniques like Raman or fluorescence spectroscopy while simultaneously lowering the limits of detection (LODs) and broadening the dynamic range of spectroscopic analysis [31, 90]. This has led to fluorescence-based POC devices, in particular, as being one of the most widely explored and currently utilized optical modalities for the detection of multiple analytes on a single test. Examples of fluorescence-based POC devices being explored include LOC microfluidic immunoassays and early disease and cancer detection through biomarker isolation [91, 92]. In terms of the equipment and optics, in recent years, there has been a significant advancement in portable handheld fluorescence spectrometers. Various commercial groups have successfully compacted benchtop fluorescence systems into portable and easy-to-use hand-held devices. Cutting edge advancements in affordable optical sensing and imaging have also been achieved, most notably with the development of cellphone-based designs [58, 93] that can have major healthcare impact in low resources settings including the developing world.

Fluorescence POC devices do have some limitations, particularly relative to their multiplexing capabilities due to the dyes’ broad emission spectrums, which can overlap when using multiple dyes. Furthermore, the need for different excitation wavelengths to excite multiple dyes can pose additional challenges [94, 95]. The use of dyes or reporter molecules has also become popular in other spectroscopic platforms beyond fluorescence. Most interesting has been the use of multiple fluorescent dyes, photosensitizers, or other reporter molecules with Raman spectroscopy. The spectra of the same dye reporter obtained using Raman produce spectral peaks with a full width half maximum 10–100 times narrower than the peaks typically observed using fluorescence [27]. These thin spectral bands provide the capacity for SERS to be used for multiplexed detection of several biomarkers from a single measurement [96].

3 Surface enhanced Raman spectroscopy (SERS) and its potential benefits

Raman spectroscopy provides a “chemical fingerprint” as it relies on loss (Stokes) or gain (anti-Stokes) in energy of an inelastically scattered photon due to a molecular
vibrational event. Traditional spontaneous Raman scattering is intrinsically inefficient compared to elastic Rayleigh scattering, as it relies on the polarizability, or Raman cross-section, of the analyte. Even when probing analytes such as MBA, DTNB, and BPE, which have aromatic rings (containing benzene) whose pi-bonds can be easily delocalized around the ring, producing distinct vibrational modes [97], the probability of a photon to be scattered inelastically is only ~1 in 10^8 [98].

SERS is one technique that can enhance the Raman signal by several orders of magnitude by amplifying the electron cloud density around metallic nanostructures [99–101]. SERS signal enhancement is achieved through two distinct mechanisms: electromagnetic enhancement and chemical enhancement [100, 102–104]. For both mechanisms to occur simultaneously, an analyte must be adsorbed onto or reside very close to a dielectric (often metallic) surface [90]. Electromagnetic enhancement utilizes metallic nanostructures with local surface plasmon resonance (LSPR) wavelengths in resonance with the Raman excitation source. When this coupling occurs, plasmons (oscillating conduction band electrons) increase the local electron density, thus improving the likelihood of inelastic scatter events to occur when the analyte is near the particle surface [105, 106]. For chemical enhancement to occur, a molecule must be able to be bound directly to the surface of the metal so that there is a charge-transfer structure generated, i.e. an electron-hole pair that can mediate the transfer of energy from the metal directly to the molecular bonds of the analyte [103, 104].

Typical SERS signal enhancement factors (EF) are observed between 10^6 and 10^9 [104] with some reporting EFs as high as 10^14, thereby indicating that single molecule detection is possible [99, 107]. The dramatic enhancement seen for SERS signals makes it useful for detecting extremely low analyte concentrations. Various groups have documented the success of SERS for detecting analytes concentrations of nano-grams per milliliter. Some have shown successful analyte detection at pico-gram per milliliter concentrations or even claim single molecule detection [96, 103, 108].

3.1 Overview of SERS toward POC monitoring

Given the potential advantages for the SERS approach listed above, several groups have published research documenting biomarker or analyte detection using SERS platforms ultimately aiming toward improving a POC technology [101]. SERS approaches have been developed for targeting tumors [109], diagnosing malaria [21, 27], identifying bacterial meningitis [110], and many other applications. This section will provide an overview of several biotechnologies that use SERS with a focus on colloidal particles. Benefits and limitations of each approach will also be presented in the interest of the future development POC technology.

3.2 Nanoparticles as SERS substrates

Starting from the 1970s, colloidal nanoparticle suspensions have served as one of the most popular SERS substrates, due to their relatively basic synthesis process requiring only a silver salt and a reducing agent to produce a metallic sol, with submicromolar detection limits. One of the first examples was demonstrated by Lee and Meisel using silver nanoparticles and carbocyanine dyes in 1982 [111]. In this work, they also observed the extra enhancements achievable when combining SERS with resonance Raman (RR) as was seen by Albrecht and Creighton using rhodamine in 1977 [112]. It was observed that by choosing analytes with molecular absorption near the excitation wavelength, as is common for many fluorophores and cell staining dyes, additional Raman signal enhancement occurs. When the resonant dye molecule is bound to a plasmonic material, both the electromagnetic and chemical enhancement mechanisms discussed previously occur and are coupled; the resulting technique is referred to as surface enhanced RR spectroscopy (SERRS). Thus, maximal signal enhancements are achieved by tailoring the resonance of the metallic substrate with that of a chromophore that is also resonant with the excitation light and capable of binding directly to the particle surface.

Not long after in 1989, Rohr, Cotton et al. revealed the first SERRS immunoassay capable of monitoring antibody/antigen interactions for the biomarker human thyroid stimulating hormone (TSH) [113]. Their one-step, no wash, sandwich-type assay used a resonant dye to indirectly monitor TSH in the physiological range, thus validating the potential of SERS to enhance and improve diagnostic assays. A decade and a half later, Nie and Emory were able to achieve SERRS enhancements of up to 10^8 when probing individual rhodamine 6G molecules on single silver nanoparticles [114], thus enticing future investigators to utilize this technique for POC applications that require ultra-low LODs.

Many early approaches used planar arrays of plasmonic nano-rough structures to facilitate the translation of SERS to a widely accepted, commercially viable platform diagnostic technology. Planar SERS substrates that provide maximum enhancements by forming nanogaps
between metallic particles, where plasmons can couple and generate defined “hotspots” [115]. These hot spots have a strong spatial and local field dependency that tend to occur within aggregates, at sharp edges, and areas of large curvature of metallic nanostructures (on the order of 10–100 nm) [116–118]. Due to the difficulty in reproducibly preparing nanostructures, many planar SERS substrates must be synthesized using a variety of sequential complex photolithographic techniques borrowed from the silicon wafer industry. These methods include techniques such as using electron beam deposition (EBD) to create the nanorough surface itself [90, 119], using EBD to produce “nanostamps” [120], combining nanoimprint and copolymer lithographic techniques [121], using self-assembled nanoparticles (SAMs) [122, 123], and many others using non-spherical particles such as Au- and Ag-coated nanorod arrays [124, 125]. These techniques, specifically EBD, produce high-resolution nanostructures that are reproducible within a wafer; however, there is a tradeoff with high reproducibility and low enhancement factor (10^2–10^4) [126]. Due to the difficulty of fabricating well-controlled small gaps or complex geometries, nanomaterials have also been cast into thin films [127–129] or deposited onto two-dimensional nanostructured arrays [130, 131] to create hybrid substrates and improve the efficiency and density of SERS active sites.

However, an ideal SERS substrate should not only deliver maximal enhancement but also provide a reproducible and uniform SERS response, have a stable shelf-life, and be easily fabricated [132–134]. Although planar SERS substrates are commercially available, scanning electron microscopy/transmission electron microscopy images provided by distributors often reveal a lack of batch to batch reproducibility, making the formation of clinical calibration curves very difficult. Additionally, due to the expensive nature of using a clean room in order to produce reproducible planar SERS substrates, each individual test would cost far more than the ~$1–10 limit for POC technologies. Therefore, the focus in the following sections is on SERS substrates that utilize colloidal plasmonic nanoparticles, as they are arguably the simpler SERS substrate to produce without the need for complex equipment or extensive training.

### 3.2.1 Developing colloidal SERS substrates

Colloidal SERS is most commonly achieved using silver and/or gold nanoparticles between 20 and 200 nm, as they exhibit unique and tunable optical properties to facilitate the plasmonic coupling event required in SERS sensing. The nanoparticle size [135], shape [136], dispersant [137], and particularly the dielectric properties of the metal all strongly affect a colloid’s extinction spectra and SERS capabilities. To ensure a colloid’s SERS enhancement factor is uniform throughout the suspension, it is also required that the nanoparticles be somewhat monodisperse (PDI < 0.300) [138]. Spheroids exhibit LSPRs that are typically within a ±120-nm window of the most commonly used laser sources: 532 nm, 633 nm, and 785 nm (Figure 1 [139]). Commonly, SERS enhancements are achieved by tuning the Raman excitation wavelength close to the intrinsic LSPR peak of the colloid or by causing the colloid’s LSPR to shift into resonance with the laser by altering the particle’s properties [140]. This has been realized using controlled salt-based aggregation, mechanical trapping or centrifugation of particles, creation of core-shell particles, or binding event that results in nanoparticle assembly formation, for example, using DNA hybridization to template core-satellite formation as described extensively by Mirkin and colleagues [141–143].

![Figure 1: Photographs (A) and extinction spectra (B) of various 60-nm metal nanoparticles (AgNPs, AuNPs, and Au/Ag nanoshells) in water. LSPR peaks are observed at 430 nm, 540 nm, and 630 nm, respectively. Reprinted with permission from [139]. Copyright 2013 Royal Society of Chemistry.](image-url)
3.2.2 Label-free colloidal SERS

As Raman provides a chemical fingerprint of the probed sample, it may seem intuitive to detect disease identifying changes in an isolated biomarker’s conformational structure by looking at the Raman modes coming from the analyte itself. However, the specificity or ability to uniquely measure a desired biomarker in complex biological samples is a key factor for translating SERS technologies to the POC and could inhibit simply looking at the analyte itself. Thus, for label-free methods such as monitoring the intrinsic spontaneous Raman (SR) or stimulating the resonance Raman modes of the analyte (Figure 2), the biomarker must be a pure sample and be isolated from the complex biological sample using extraction techniques such as HPLC or an immunoassay, or the biomarker itself must exhibit vibrational modes that can be uniquely pulled out from the modes inherent in the background media [145]. Bringing these purification steps to the POC often requires immobilization of affinity ligands such as antibodies or DNA aptamers onto well plates, scintillation vials, or glass slides using basic click chemistry [146]. The downside of these ELISA or extraction kit-style designs is the complexity (i.e. not user-friendly by the WHO-defined “ASSURED” criteria) including required user intervention for washing steps to remove optically or biochemically interferent components of the sample prior to SERS analysis.

For example, our group was able to observe structure changes in the SERS spectra of the biomarker β-amyloid absorbed onto aggregated gold nanospheres [147] in a pure sample that could be suggestive of Alzheimer's disease. A second example was conducted first by Feng et al. [148] and later Wang et al. [144] using two abundant serum proteins, albumin (Figure 2) and globulin, to detect colorectal cancer. In Wang et al.’s work, the two serum proteins had to first be purified from over 200 healthy and cancerous human serum samples. Protein samples were then added directly to hydroxylamine silver nanoparticles, and acetic acid was used to aggregate silver nanoparticles to increase the magnitude of the SERS enhancement. SERS bands were assigned to verify specific biomolecular contents of the proteins and to predict protein secondary structural changes that occur with colorectal cancer progression using the difference of the SERS spectra between healthy and cancerous samples. Principal component analysis and linear discriminant analysis were required to be used to assess the capability of this approach for identifying colorectal cancer, demonstrating a diagnostic accuracy of 100% for albumin monitoring and 99.5% for globulin. Additionally, both the albumin and globulin partial least squares (PLS) models successfully predicted the unidentified subjects with a diagnostic accuracy of 93.5%.

These results suggest that SERS analysis of serum proteins have the potential to be a sensitive and clinically powerful means for disease detection. However, simple direct sensing efforts like these still struggle to fully translate to the POC, as they require complex sample preparation to be performed before SERS analysis. Thus, these methods are not user friendly as noted above, are time consuming, involve complex statistical analysis or peak assignments, and require too many separate pieces of laboratory equipment to be fully implemented at the patient bedside.

3.3 Molecularly mediated colloidal SERS

Indirect sensing using an assay whose SERS response is facilitated by a molecular binding event, particularly one that involves the biomarker itself, has emerged as...
an efficient approach to colloidal SERS. Specifically, SERS-active nanoprobes have the potential to enhance specificity [149]. In general, they can involve one or more nanoparticles conjugated to (1) a highly polarizable Raman reporter molecule, (2) an affinity ligand such as an aptamer or antibody, and (3) a steric or electrostatic capping agent for stabilization in high salt environments [150, 151].

Oligonucleotides, antibodies, protein antigens, small molecules, and dyes can all be immobilized onto metallic nanoparticles using thiol end groups, bifunctional PEG linkers, or sequential click chemistry [152–155]. Extensive work has been done by groups such as Graham et al. who utilize various oligonucleotide and resonant dye-coated nanoprobes to form SERRS active nanoassembly complexes for multiplexed DNA detection [156]. In most cases, the nanoassembly detection modality involves the SERRS active particles’ LSPR shifting in or out of resonance with the excitation source. This is caused by hybridized DNA linking of the nanoparticles in close proximity in order to share conduction band electrons and red-shift their extinction spectra. They, thereby, jump in SERS intensity, without causing irreversible aggregation (Figure 3). The group has translated their “SERS-on” techniques for a variety of DNA, protein, and small molecule sensing applications, most recently by Mabbott et al. for monitoring four fungal probes in a multiplexed fashion [157], by Simpson et al. using a biomimetic glyconanoparticle assay for ultrasensitive (ng/ml) quantification of cholera toxin B-subunit [158], and by Gracie et al. for the simultaneous detection of two meningitis bacterial DNA biomarkers extracted from cerebral spinal fluid (CSF) clinical samples [110, 159].

Another approach uses only one colloid without aggregation, relying solely on small molecule binding to either competitively displace an aptamer tagged with a Raman dye molecule in a “SERS-off” configuration (Figure 4). Chung et al. utilized this “SERS-off” moleculearily mediated SERS methodology and used a partial complimentary sequence to immobilize an ssDNA aptamer onto Au/Ag core-shell nanoparticles. This method proved to be sensitive down to the 10-fm range for BPA-spiked tap water, over a total dynamic range of 10 fm–100 nM [160]. The authors of this work acknowledge that this LOD is two or three orders of magnitude lower than that reported for other BPA sensing techniques but may possibly be higher if the samples were in complex biological media. It is noteworthy that the total detection time was estimated to be about 40 min including both the reaction between aptamer and BPA (30 min) and detection (10 min), making this option good for supplementing rapid diagnostic tests (RDTs).

3.3.1 Magnetic approaches for colloidal SERS monitoring in complex samples

One obstacle preventing the translation of molecular diagnostics using SERS at the POC is the lack of simple methods that can be integrated into portable platforms, for instance, without the need for complexity such as sample washing steps. One potential technology to overcome
this challenge is the use of magnetic approaches including microbeads and superparamagnetic nanoparticles (SPIONS) [161]. These are easy to manipulate with small permanent neodymium magnets held at the side of a vial, well plates, capillary tube, microfluidic channels, or even inside cells [162]. When functionalized with sensing ligands, this allows for faster, more automated washing steps while also preventing sample sedimentation often seen with repeated centrifugation [29, 163]. Magnetic nanoparticles can also provide a plasmonic response when coated in gold or silver [164], therefore improving SERS enhancement capabilities [165–167].

Many groups have facilitated this technique for improving clinical chemistry techniques recently, such as Wang et al. who used aptamers immobilized onto silver-coated magnetic nanoparticles along with a secondary SERS active gold nanoprobe coated with another aptamer to capture and quantify bacterial cells down to 10 cells/ml [168]. Ge et al. used a similar sandwich binding approach but with antibodies in place of aptamers for the detection of the ovarian cancer serum biomarker human epididymis protein 4 (HE4). They not only were able to demonstrate fg/ml LODs and a dynamic range of 1 pg/ml to 10 ng/ml but also demonstrated that the assay particles could be washed and reused at least five times in their efforts towards developing easy to use diagnostic kits [169].

Tuan Vo-Dihn’s group has also developed a sandwich-type SERS assay, relying on specific DNA hybridization to capture ultrabright SERS nanorattles onto magnetic microbeads [27]. As shown in Figure 5, nanorattles are core-shell silver particles with RR reporters loaded in the gap space between the core and the shell. The DNA probes are coated on the shell surface, thus acting as the SERS tags for signal detection. After hybridization, a magnet was applied to the bottom of the well to both remove unbound nanorattles and to concentrate the hybridization sandwiches at a localized detection area for SERS measurements. Probing for two specific DNA sequences of the malaria parasite Plasmodium falciparum, one mutated and the other wild type, it was found that SERS could detect malaria DNA down to 100 am. As the mutant sequence translates for resistance to artemisinin drugs, single nucleotide polymorphism (SNP) discrimination of wild-type malaria DNA and mutant malaria DNA was also demonstrated. Their results show the potential for molecularly mediated SERS to differentiate small mutations in infectious pathogens with far greater sensitivity than current methods, an important factor for global health applications.

Another relevant magnetic-based method for potential use in SERS sensing was demonstrated by He, Li, and Hu with an aptamer recognition-induced target-bridged SERS assay based on magnetic chitosan (MCS) and silver/chitosan nanoparticle (Ag@CS NPs) binding [170]. A single aptamer target nanoparticle was used for the detection of three different types of protein, benefiting from the highly specific affinity of aptamers and biocompatibility of chitosan (CS). MCS coated in various antibodies or aptamer act as capture probes in the triple sandwich assay format.
shown. The sandwich complexes of aptamer (antibody)/protein/aptamer were first mixed with complex biological mediate and separated from biological samples after the reaction proceeded by magnetic manipulation with a permanent magnet under a glass slide. SERS signals were collected after washing the complexes, and the protein concentrations indirectly correlated with the number of Raman reporter molecules left after washing. To demonstrate the translatable stability of this method, three different proteins – thrombin, platelet-derived growth factor (PDGF), and immunoglobulin E (IgE) – were investigated. The CS shell demonstrated enhanced stability for longer shelf life and prevention of signal drift due to loss of Raman reporter.

Like many colloidal nanoparticle assays, this method avoids slow diffusion limited kinetics problems observed for solid SERS substrates. The feasibility of this method for potential use at the POC was shown with PDGF BB in clinical serum samples, with an LOD of 3.2 pg/ml. The prediction results obtained from human serum of healthy patients vs. cancer patients using the proposed SERS method correlated with traditional ELISA results while the SERS method expanded the linear range.

### 3.3.2 Combining SERS-based immunoassays and ELISA

SERS nanoprobes have recently been used to improve detection capabilities of immunoassays and have the potential to rival the popular enzyme-linked immunosorbent assay (ELISA) techniques. Combining SERS and ELISA, aka “SLISA”, has proven to be an effective method for improving the LODs due to the intrinsic enhancement capabilities of SERS, the ability to speed up the assay reaction times due to the 3D architecture of functionalized colloidal nanoparticles, and the ability to capitalize on the narrow spectra bands obtained with Raman for improving the multiplexing capabilities of traditional immunoassays [171]. Bhardwaj et al. directly compared the capabilities of SLISA and ELISA assays for the measuring RAD54 stress-marker proteins. They found that SLISA has similar accuracy as ELISA but improves upon the indirect enzyme-based method by being reusable, faster, more direct, and easier to use. SLISA was also five times more sensitive than ELISA while providing qualitative information on the immunosensor’s chemical characterization and antigen-antibody binding. This allows direct detection with less uncertainty, which is a stringent limitation of all label-based biosensor technologies, including ELISA [172].

One example of a biomarker candidate for SLISA is the hormone estradiol (17β-estradiol, E2), a critical serum protein in sexual development. The E2 levels are especially low (<10 pg/ml) in prepubertal girls, and current clinical detection methods are insufficient for accurate assessment of E2 at these ultralow concentrations. In a study conducted by Choo’s group, a new E2 sensor was introduced using a magnetic capture bead SERS immunoassay detection platform [87]. The work was based on their previous work that validated the technique for use with clinical samples for the early diagnosis of arthritis [173].

The system involves a competitive binding assay with reagents immobilized onto magnetic beads to assist with automated wash steps and also to enhance the SERS response through magnetic aggregation in a glass capillary tube (Figure 6). Their SERS assay was tested with 30 blood samples to assess its clinical feasibility, and their prediction results were compared to those obtained using a commercially available chemiluminescence immunoassay. The commercial immunoassay failed to quantify E2 serum levels lower than 10 pg/ml, but the LOD of E2 using the novel SERS-based assay described in this study was an order of magnitude lower at 0.65 pg/ml. This verified that SLISA-based methods have a strong potential in
the early identification of biomarkers due to their exceptional analytical sensitivity.

3.3.3 Dual modality colloidal SERS

Beyond SLISA, another emerging trend in colloidal SERS is the utilization of dual optical modality approaches. For example, many colloidal SERS assays also intrinsically exhibit a colorimetric response and dual sensing can facilitate simple yes/no readouts [174, 175]. Researchers have also combined SERS with fluorescence to provide additional visual confirmation of binding results in a multiplexed format [176]. As more methodologies emerge and combine, the benefits of SERS will only be expended even further.

4 Implementation of SERS POC technology using different fluidics platforms

The aforementioned advancement in SERS assays for the detection of biological analytes in complex media has supported its potential use in POC platforms for the detection and monitoring of different diseases. This section provides an overview of the two major platforms, microfluidics and paper-based fluidics. Both are being invested by several groups in the transformation of SERS assays towards future POC biotechnologies.

4.1 SERS combined with microfluidics

Microfluidics is the science and technology of manipulating and controlling fluids typically in the range of microliters to picoliters using microchannels [177]. The use of microfluidics for analytical biosensing has the potential to not only facilitate the assay procedure but also improve assay results [107, 177]. In particular, advances in the microfluidics technology field have contributed to the development of LOC biosensors. The use of microchannels, microvalves, micomixers, and micropumps has allowed the creation of small chips that can potentially perform all of the functions needed in an immunoassay procedure [177]. The main advantages of microfluidic based biosensors are that they can measure minimal sample volumes and potentially eliminate the need of user input in the process. Also, they usually have short times for analysis, which could be very important for POC diagnostics.

In common immunoassays procedures, the user usually has to dispose of the samples, add reagents, wash wells, mix solutions, and take measurements. The use of microfluidic based biosensors can eliminate many of these steps and thus reduce the possibility that human error can affect the measurements. This can potentially be translated into improved sensitivity, precision, ease of use, rapid results, and minimal amount of sample needed [178].

Several groups and multiple reviews have incorporated microfluidic technology with SERS-based assays to create sensitive sensors for potential POC applications [107, 178–180].

4.1.1 Mixing in the microchannel

The ability to reproducibly mix the components of an assay is essential for the appropriate functioning of a diagnostic test. The SERS-based assays that use functionalized colloidal nanoparticles typically have to be thoroughly mixed with the analyte of interest and other components to allow them react and interact to produce accurate results. Therefore, one focus in the development of a microfluidic SERS-based assay is the mixing section.

For example, Chon et al. developed a SERS-based microfluidic sensor that serially dilutes the target marker, mixes antibody-conjugated hollow gold nanospheres (HGNs) and magnetic beads, and traps the magnetic complexes with different structures. In the microchannel, a groove-shaped mixer was incorporated to improve the mixing efficiency [181]. Figure 7 shows the microfluidics design.

Wilson et al. described the development of a microchannel that uses a mixer to enhance the contact between silver colloid and an analyte. They were able to detect it with a sensitivity that was an order of magnitude greater than without using the chip [182]. In another approach, Quang et al. created a microchannel with a micropillar array to achieve efficient mixing and produce reproducible SERS detection. This microchannel allowed the detection of dipicolinina acid (DPA) and malachite green (MG) with estimated detection limits of 200 ppb and 500 ppb, respectively [183].

In another example, Geo et al. described a microfluidic based biosensor to detect the prostate-specific antigen (PSA) cancer biomarker [30]. They created a SERS-based magnetic immunoassay on a microfluidic chip, as can be observed in Figure 8. The microfluidic channel was designed to generate and mix microdroplets with the
assay reagents. Inside the droplet, the antigen and the antibodies reacted to form sandwich immunocomplexes.

A magnetic bar embedded on the channel separated the magnetic immunocomplexes from the SERS nanotags unbound to the magnetic beads. The droplet was split into two parts with Y-shaped channel bifurcation. The fission created two droplets, one with the magnetic immunocomplexes and the second one with the unbound SERS nanotags. The SERS signal of the droplet containing the SERS nanotags was measured and analyzed. The LOD of the SERS-based microdroplet sensor was estimated to be below 1 ng/ml, which is lower than the value used in common diagnostics of PSA [30].

4.1.2 Nanoparticle aggregation and SERS substrates in microchannels

SERS can be used to develop specific and sensitive biosensors. However, a main challenge of this modality is to obtain reproducible results from measurements. The reproducibility of SERS measurements is affected by different factors such as the type of substrate used, the aggregation method, and the inhomogeneous distribution of molecules on the metallic substrate [107]. Thus, controlling the aggregation of colloid used in SERS sensors has been a main focus, as the development of this technology. A reproducible aggregation of nanoparticles or controlled deposition of enhancement structures on a surface can be translated into a consistent SERS enhancement. As a result, several groups have tried different approaches to control the aggregation of nanoparticles or to produce reproducible SERS substrates on surfaces with defined enhancement spots [107].

Wang et al. developed an optofluidic device with a microchannel-nanochannel junction to trap and assemble nanoparticles into SERS active clusters by using capillary force (Figure 9). This cluster provided an electromagnetic enhancement factor of about $10^8$. However, they reported...
a SERS enhancement reproducibility of ±10% (device to device) when 83 nm of adenine was used [184].

Yazdi and White described another aggregation method by forming a 3D nanofluidic network with packed nanoporous silica microspheres in a microfluidic channel [185]. This matrix trapped silver nanoclusters and adsorbed analytes into the SERS detection area. With this approach, a concentration of R6G of 400 am was detected. A multimode fiber optic was also integrated in the channel, which eliminated the need for optical alignment.

Magnetic sections have also been used to aggregate nanoparticles for SERS measurement. Gao et al. demonstrated an assay to detect the anthrax biomarker poly-y-D-glutamic acid (PGA) on a microfluidic chip (Figure 10). In this assay, PGA and PGA-conjugated gold nanoparticles competed for binding sites on anti-PGA-immobilized magnetic beads. The magnetic immunocomplexes were trapped by yoke-type-solenoids embedded on the microchannel where the SERS signals were measured. The assay estimated LOD was 100 pg/ml [186].
Instead of aggregating nanoparticles to create the SERS enhancement, other groups have developed microfluidic devices with nano-rough substrates already incorporated. For example, Liu et al. described the creation of nanowell structures on PDMS. A thin Ag film was deposited on the nanowells to create SERS active sites. The SERS spectra of Rhodamine 6G and adenosine were measured with the microchannel. The SERS enhancement on the nanowell-based Ag SERS substrate was more than 10^7 times higher than on a smooth Ag layer on PDMS [187]. A similar microchannel was reported by Oh et al. where plasmonic nano-probes with hotspots were selectively patterned on PDMS microchannels. This microchannel enabled solution-phase SERS detection of small molecules [188].

Most microfluidic devices are made from polydimethylsiloxane (PDMS) because the fabrication of channel systems is straightforward and the channels are flexible. PDMS channels can also be combined with SERS substrates prepared on a surface, such as glass [187].

As mentioned, the advantages of using microfluidics for potential POC diagnosis include the use of minimal volumes; the ability to control the sample and perform steps such as mixing and washing automatically; the ability to increase the SERS substrate reproducibility, which improves precision; and the ability to rapidly detect biomarkers with high sensitivity. However, some of the disadvantages of microfluidic based sensors can be the cost to manufacture the chips and, in some cases, the need for external devices to control fluid flow. Thus, new technologies such as paper-based SERS biosensors are being developed to address these issues.

4.2 Paper-based SERS platforms

There are several different fabrication techniques for paper-based sensing microfluidics reported in the literature [189–192]. However, for this review, only techniques utilized in combination with SERS-based detection towards POC will be discussed.

Paper-based SERS is attractive due to its low cost, simplicity, ability for multiplexing, and reduction in analysis time. Typically, these “paper fluidics” are cellulose based, allowing for the flow or imprinting of nanoparticles embedded within its matrix. This technique has been adapted from chromatography where samples can be separated and analyzed based on size, shape, or surface charge, which allows for separation, detection, and analysis, all on a single platform. This section focuses on different fabrication techniques and monitoring approaches for paper-based SERS detection that have the potential to be adopted for POC applications.

4.2.1 Filter membrane-based SERS with a syringe

Filter membrane-based SERS detection utilizes inexpensive tools such as a disposable syringe, filter holder, and filter membrane. The basic concept involves pre-wetting the filter membrane with an organic solvent such as ethanol, passing colloidal (Au or Ag)-coated nanoparticles through the filter membrane via the syringe and then passing the sample through the filter (Figure 11) [193]. The colloidal particles are coated with the desired target probe that interacts with the sample. The filter membrane allows for the aggregation of these particles for SERS detection via portable spectrometer after removal and drying of the membrane. Research groups have utilized this quick and easy method for detection of chemical and biological entities such as melamine and malathion [193], Escherichia coli [194], and other toxins or pathogens in food [195]. Due to the simplicity of this approach, the technique can be utilized in on-site diagnosis. However, White’s group reported that this technique was shown to be two to three orders of magnitude less sensitive than other paper-based SERS methods [193].

4.2.2 Dip coating paper-based SERS

Filter paper can also be used to absorb nanoparticles via immersion of the filter paper into solutions of colloidal nanoparticles. Typically, filter paper such as Whatman®...
is submerged for a period of time in solution of colloidal nanoparticles. The paper is then removed and allowed to air dry. The particles are retained within the filter’s matrix via adsorption due to van der Waals forces and hydrophobic interactions between the nanoparticles and fibers [196]. Groups such as Cheng et al., Liu et al., and Ngo et al. have utilized this technique to detect tyrosine [197], oral cancer cells [198], and antigen [199], respectively. Cheng et al. reported that the use of filter paper to detect tyrosine provided 50 times more SERS signal and a detection limit of 625 nm [197]. This approach provides an easy fabrication method of the SERS substrate and can be widely used as a swab or dipstick to collect samples.

4.2.3 Printed paper-based SERS substrates

Commercially available inkjet printers can be reengineered for use in printing highly concentration colloidal nanoparticles on specific regions of the paper or for patterning hydrophobic barriers to direct the flow of the fluid. Typically, either a thermal or a piezoelectric printer is used depending on the thermal sensitivity of the SERS substrate and/or the solvent being used.

In terms of nanoparticle printing, Yu and White demonstrated that colloidal silver nanoparticle arrays can be printed on cellulose-based paper to form aggregates within its matrix using an EPSON Workforce 30 inkjet printer [200]. The printed paper was then cut into a specific shape and dipped into a solution containing the analyte of interest. After time was given for the liquid to be wicked and allowed to travel to the assay region, a fiber optic, portable spectrometer (excitation wavelength of 785 nm and laser power of 17 mW) was used to detect varying concentrations of drugs and pesticides on the nanogram scale [200, 201]. This technique provided high density aggregation of the colloidal nanoparticles to enhance the SERS signal. However, this printing approach can be difficult to produce repeatedly. Other limitations also include complications in modification of the printer and problems with nozzle clogging.

Besides using the printer to deposit nanoparticles on the surface of paper, the high resolution of inkjet printing can also be used to pattern hydrophobic borders on hydrophilic paper to create microchannels. These hydrophilic channels created within the paper are used to direct the flow of fluids through the membrane to regions of assay interaction and sensing. This is typically accomplished using a patterning agent such as wax and selectively creating hydrophobic regions. Torul et al. demonstrated the detection of glucose from whole blood by placing a droplet of blood on a gold wax printed paper containing gold nanorods [202].

SERS substrates can be screen printed on filter paper at an even lower cost than inkjet printing. This process is carried out by forcing a high concentration of colloidal nanoparticles through a meshed screen printing plate designed with a desired patterned aperture [203]. This creates multiple areas of SERS reactive regions on the paper. After drying, the paper-based SERS substrate can then be exposed to various samples and probed with a portable Raman spectrometer for analyte detection. Qu et al. used this technique to detect multiple biological analyte on a single paper with LODs ranging from $10^{-7}$ to $10^{-10}$ [203]. However, one major disadvantage to using this approach is controlling the viscosity of the colloidal nanoparticles without significantly hindering the SERS signal.

4.2.4 Lateral flow paper-based SERS

One of the earliest and most widespread POC devices is based on the concept of lateral flow and used to create off the shelf testing platforms, such as the at-home pregnancy test and glucose self-testing strips [204, 205]. In recent years, this same approach has become attractive once again to create rapid detection kits for more complex biological analytes in the early stages of disease (Figure 12) [206]. Lateral flow relies on capillary forces to move small molecules along a transport medium such as cellulose paper. By combining this technique with chromatography, more complex media can be collected, separated,
and analyzed all on one low-cost, easily fabricated testing platform. Lateral flow paper-based microfluidics eliminates the need for precise patterning or printing and reduces the cost of equipment because the flow and separation are dependent on the shape, size, and sharpness of the corners of the paper. Fu and Choo incorporated this technique to design a POC technology capable of detecting the human immunodeficiency virus type 1 (HIV-1) DNA [207]. Choi and Choo also demonstrated another clinical application of this approach for the detection of the thyroid-stimulating hormone in biological fluids in diagnosing hyper/hypo-thyroidism [208]. They reported that this approach was two orders of magnitude more sensitive than conventional colorimetric approaches. Choo’s group has also applied this technology to design a SERS-based immunoassay for staphylococcal enterotoxin B [206].

4.2.5 Current limitations of paper-based SERS microfluidics

Overall, printed paper microfluidics for SERS detection of chemical and biological analytes is an attractive technology because it offers an affordable and easy method to mass produce devices that can be used with a portable Raman spectrometer for potential on-site diagnosis. However, many of the paper-based approaches described are reasonable for detection but not yet adequate for quantification. In other words, they have been used primarily to determine whether or not the biomarker is present but rarely used to determine the repeatable concentration of the biomarker across a dynamic range. Furthermore, although the Raman spectrometers have been reduced in both size and price over the past two decades, the hand-held devices are still too expensive for delivering POC devices. If portable Raman spectrometers could be mass produced for high volume POC applications, rather than primarily used as research tools as they are now, it is reasonable to assume the cost and size will continue to be reduced.

5 Conclusions

POC technologies that follow the WHO’s “ASSURED” guideline can offer many advantages over current bench-top lab-based measurements and provide a means for RDT. In this review, SERS has been explored as a dynamic technique for POC monitoring because of its high sensitivity (up to fm detection limits) and multiplexing capabilities. Many SERS-based assays and platforms are currently under development. This review focused on comparing these systems in terms of the synthesis, functionalization, and utilization of plasmonic nanoparticles as the SERS substrates within different environments including micro-wells, microfluidics, and paper-based platforms. In particular, device complexity can be reduced by coupling the SERS-based substrates with low cost, easy to fabricate, paper fluids.

While the high sensitivity and multiplexing ability of SERS hold promise for its use in POC diagnostics, it still fails to produce signals as robust and repeatable as current gold standard assays. Additionally, researchers rarely present methodologies that can be translated across multiple biomarker types or for analytical ranges spanning over several orders of magnitude. However, advances in more specific and robust capture ligands such as DNA aptamer-coated nanomaterials offer a promising emergent solution. Furthermore, SERS is also limited by the high cost of portable Raman readers although hand-held systems have been significantly reduced in size and cost over the past decade. Overall, the promise for SERS to be used for POC monitoring will rely on overcoming the barriers through further advancements in the assays, platforms, and more dedicated, cost-effective, Raman readers.

Acknowledgments: The authors wish to acknowledge the financial support of the National Institutes of Health (2R44ES022303). Furthermore, we would like to thank Samuel Mabbott for his helpful discussions and review of this manuscript.

References


