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RESEARCH PAPER

A Review of Paper-Based Substrates as Surface-Enhanced Raman Spectroscopy (SERS) Biosensors and Microfluidic Paper-Based SERS Platforms

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Abstract

Raman spectroscopy is an important method for identifying molecules and has many uses in determining the chemical and structural properties of different materials. Despite the structure of the intelligently enhanced substrates used in laboratory research, the development of a simple, flexible, and cost-effective substrate is also important for enhancing the application of surface-enhanced Raman spectroscopy (SERS) in practical analysis. Recently, paper has been considered for the fabrication of flexible SERS substrates. Compared to other SERS substrates, paper substrates have the unique advantage of strong mechanical properties, various components, and adjustable pore size. These features give many advantages to paper-based substrates for SERS analysis in practice, such as low-cost and straightforward substrate preparation, high efficiency, separation, and detection methods. Therefore, paper-based substrates in SERS analysis have been promising in applications such as environmental monitoring, food safety with high sensitivity and efficiency, etc. This review presents a summary of the research related to paper involving SERS analysis. First, a brief introduction to understanding its background is provided, followed by a brief history of the paper-based substrates. Then, the preparation of the paper-based substrate and the role of the paper are summarized, and the applications of paper-based SERS substrate in the analysis are presented. Then, in a separate section, several studies reported in the field of microfluidic paper-based SERS platforms are reviewed. Finally, the challenges and perspectives of this issue are discussed.

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1. Introduction

One of the efficient methods for studying low concentrations and detecting even single molecules is the surface-enhanced Raman spectroscopy (SERS) method [1]. The SERS has become one of the most versatile and robust analytical techniques due to its extremely high sensitivity, unique fingerprint specificity, and non-destructive analysis in molecular assays [2]. These unique advantages make SERS a promising tool in various branches of analytical sciences, such as environmental monitoring, food analysis, surface analysis, disease diagnosis, and other related fields [2-4]. The efficient enhancement of SERS substrates plays a vital role in achieving these advanced applications. So far, many substrates have been fabricated with different components, structures, and arrangements, and these studies are still ongoing. The search for new materials (semiconductors [5], graphene [6], organic metal framework [7], etc.) to achieve an enhancement in Raman signal is always an important topic in SERS research, and several excellent works have been published on this subject [8–10]. Although many advanced Au/Ag micro/nanostructures or their combinations with other materials have been developed for SERS analysis, most are just prototypes used in laboratory analysis. Fabrication of these substrates often requires complex equipment or high skills or costs that make mass production difficult and therefore not available for practical analysis. In addition to the discovery of new components, the development of flexible and easy-prepared substrates has become a new trend in recent years in the demand for practical analysis [11-13]. In this regard, the paper-based SERS substrate has attracted much attention. Chemically produced gold or silver nanoparticles can be adsorbed on or assembled on a flexible matrix to form a flexible substrate. This type of substrates can have acceptable mechanical strength, show high efficiency in adsorbing and identifying targets on uneven surfaces, and be cut to the desired size and shape and easily integrated with other structures or devices. It is a reason that flexible substrates are important for advancing real-world applications of SERS technology. Currently, flexible SERS substrates are mainly based on paper [14-15], flexible polymers [16], carbon nanotubes, and graphene materials [17]. Moreover, flexible SERS substrates have been extensively developed, and their progress has been summarized in several well-reviewed studies published in recent years [11-13, 18,19]. The paper-based SERS substrates have been developed with great foresight and have attracted many researchers' attention in this field. These systems are promising in environmental monitoring, food analysis, and other related fields [20]. Although previous works have provided a review of the applications of SERS method for chemical and biological analyses [21], there is not a comprehensive review in the field of paper-based SERS platforms published so far. Therefore, a classified review was needed in this field. For this reason, to better understand the origins and development of paper-based SERS analysis and to further promote research in this area, the present study reviews the definitive and recently developed applications of paper-based SERS platforms for biochemical characterization of important compounds (especially medical and biological) and the detection mechanisms, direct and indirect fabrication methods for the identification of biological materials, applications of biosensors, mechanisms of amplifiers and SERS-based biosensor structures to detect biomolecules, and the theoretical foundations of the SERS effect, and microfluidic paper-based SERS platforms are addressed, and the future applications of SERS in this field are discussed. The authors hope that this review article will play a positive role in promoting SERS as a competitive tool in rapid and accurate practical analysis.

2. Applications of SERS spectroscopy as a biosensor for the detection of medical and biological materials

Films composed of noble metal nanoparticles (typically gold (Au), silver (Ag)) have already gained considerable popularity and interest in scientific research in the field of nanotechnology due to their interesting optical properties and surface plasmon resonance. In noble metal nanoparticles, the displacement of electrons from their equilibrium position leads to optical enhancement, and they have potential applications in many different fields such as biosensors [22, 23], solar cell design [24], and SERS [25]. Noble nanoparticles have extraordinary properties, and based on this, many existing methods for making SERS biosensors have been proposed in the last few years [26]. Certainly, such methods can be used for the fabrication of SERS biosensors. Over the past few decades, metals such as Au, Ag, Cu, and Pt have been used to observe the SERS phenomenon in various experiments. The successful application of metal nanoparticles in SERS depends on the characteristics such as morphology and nature of the metal used as the substrate. Silver and gold are the most widely used metals due to their wide plasmon resonance in the Vis-NIR region, high stability, and easy preparation [27]. Metal nanoparticles placed on the substrate enhance the analyte Raman spectrum. The SERS is a selective and sensitive method in which the Raman scattering is enhanced for molecules adsorbed on metal nanoparticles. Using this method, in addition to analyzing molecular structures, information about molecule adsorption and the process of molecule interaction with the substrate surface

can be obtained [28]. Gold and copper surfaces were also tested so that such metals can increase the Raman spectrum, but due to the high stability of gold and silver, these metals are often used [29]. For the rapid and accurate study of biological and chemical samples, various substrates and methods were used to make SERS-active substrates. These substrates are made in two ways: physical and chemical [30, 31]. Biological species and materials can be easily identified using the SERS biosensors [32]. Various techniques, such as HPLC [33], Tandem Mass Spectroscopy (TMS) [34], Gas Chromatography-Mass Spectrometry (GC-MCS) [35], amperometry [36], chromatography [37], Magnetic Resonance Spectroscopy (MRS) [38], and SERS are used to measure biological material and molecules [39]. The mentioned techniques are less sensitive than the SERS method, and their application requires high costs. Raman spectroscopy is a convenient way to identify combinations of various materials, including biological species; however, the Raman signal of species, especially at low concentrations, is considerably low [40, 41]. In this method, by placing the species near the surface or their physical adsorption on metal nanoparticles, due to the interaction between the metal surface plasmons and the species, the intensity of the Raman signal increases. Therefore, SERS can be used for the rapid and accurate detection of biological species [42]. In addition to analytical sciences, this method is employed in medicines, the study of vitamins [43], biomolecules, viruses and bacteria [44], explosives analysis [45], drug detection [46], environmental monitoring [47], geology [48], etc.

3. Paper-based SERS substrates

3.1. Background and development of paper-based SERS substrates

The solution of gold or silver nanoparticles is often used on some substrates such as glass, slide or silicon, as the SERS substrate [48]. Despite the simplicity of direct use of a solution of gold or silver nanoparticles as a substrate, the resulting SERS signal has poor reproducibility [49]. However, designing and fabricating SERS substrates using Au/Ag requires complex skills. Therefore, a fast and simple method is needed to provide a highly active SERS substrate for sensitive and reproducible analysis. Yu and White (2012) developed the first paper-based SERS substrate [20]. They used syringes as well as paper and silver nanoparticles solutions to make paper-based substrates (Figure 1). The preparation process they used was simple, cost-effective, and fast. Paper can be used to concentrating the analyte, thus reducing the detection time and improving signal reproducibility. Therefore, such a substrate has been promising in the practical analysis of low concentrations of chemicals, and since then, further investigations have been reported in this context [50-60]. Gold or silver nanorods/nanowires [54, 55], gold/silver spherical nanoparticles [20], nanostars [50], or gold/silver-based hybrids with SiO_2 [56], activated carbon [57], and cellulose nanofibers [58, 59] were used on paper to make SERS-active substrate as a biosensor to identify a variety of biological and chemical materials [50, 54].

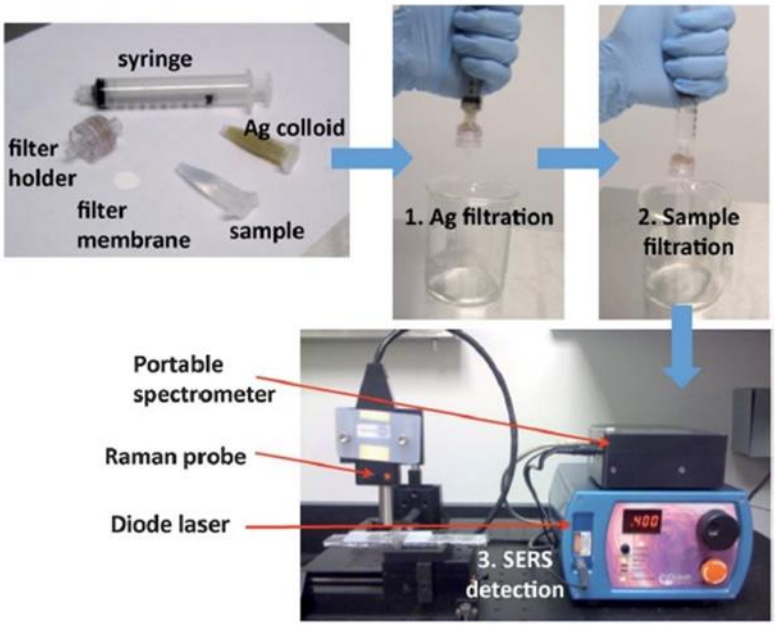

Figure 1. Preparation of SERS-active substrates using syringes by passing a silver nanoparticle solution through a filter membrane. Reprinted from Analyst, Volume 137, Issue 5, Pages 1168-1173, Yu, Wei W. and White, Ian M., A simple filter-based approach to surface enhanced Raman spectroscopy for trace chemical detection, Copyright 2012, with permission from The Royal Society of Chemistry [20].

3.2. Fabrication methods of paper-based SERS substrates

Several methods have been developed for fabricating paper-based SERS substrates. These approaches, according to the preparation process or their detection, can be divided into several types: 1) Deposition of silver nanoparticle and gold nanoparticle solutions on paper [51,54,60], which is the most used method for fabricating paper-based SERS substrates because it is rapid and simple, and the particles can be placed evenly on the substrate. 2) growing silver nanoparticles or gold nanoparticles on paper [61,62]. These two methods are extensively used to fabricate paper-based substrates [62]. However, the second way may not be as easy as the first method because the surface must be functionalized with binders to deposit gold or silver nanoparticles with high uniformity on the paper substrate. 3) Deposition of gold or silver nanoparticles on paper or other matrices using a filtration process [20]. In order to improve the reproducibility, gold or silver nanoparticles are first prepared in orderly structures of the same size and then deposited on paper [63]. By depositing gold or silver nanoparticles on paper using filtration, the repeatability of SERS signals can be enhanced [63]. 4) Gold or silver nanoparticles are first grown on porous surfaces and then deposited regularly on paper [56]. Ankudze et al. [53] deposited gold nanoparticles on flexible fibers in cotton pallets and then deposited them on paper. Lin et al. [56] deposited gold nanoparticles on a silicon substrate with a porous structure and then bonded them to paper in the form of a disk. Moreover, Jia et al. [57] deposited gold or silver nanoparticles on a silicon substrate with a porous structure and then bonded them on paper in the form of a cylindrical tube. In these cases, substrates with porous structures such as silicon, cotton fibers, and activated carbon are used to improve the adsorption capacity and Au/Ag efficiency. The manufactured product can be used as a paper substrate as a SERS-active substrate to identify a variety of biological and chemical materials.

3.3. Applications of paper-based SERS substrates for the detection of melamine, Rhodamine 6G, bacteria, and pesticides

Paper-based SERS substrates have been extensively used in the ultra-sensitive detection of organic molecules, especially some environmental contaminants such as aromatic dyes, pesticides, and antibiotics. The function of paper-based SERS substrates in large-volume samples suggests that paper-based SERS analysis is an excellent technique for environmental monitoring. The following are some of the works to be considered based on the classification of analytes.

3.3.1 Detecting melamine antibiotics using paper-based SERS substrates

The illegal use of melamine antibiotics in dairy foods can endanger human health. Accordingly, rapid and accurate detection of melamine antibiotics plays an important role in food safety control. The SERS biosensor, due to its capabilities, can be used to detect melamine antibiotics rapidly. Some paper-based SERS biosensors have been applied to monitor melamine antibiotics in various samples [20, 54]. For example, Wang et al. [64] reported a paper-based SERS biosensor for detecting R6G and melamine with great sensitivity and reproducibility. They deposited silver nanoparticles on paper that could detect R6G and the antibiotic melamine at concentrations of 5×10^{-14} M and 10^{-8} M, respectively. Paper-based SERS biosensors are difficult to oxidize; therefore, they are stable for approximately two months.

3.3.2 Detection of Rhodamine 6G using paper-based SERS substrates

Rhodamine 6G (R6G) is one of the most widely used analytes as a reference for the investigation and development of SERS substrates. Wu and White et al. used R6G to test the paper-based substrate as a SERS biosensor [20]. In their study, the paper-based SERS biosensor detected up to 10 nm of R6G and had a significantly high reproducibility. By coating AuNR on cellulose nanofibers and bonding it to paper, Zhang et al. used SERS biosensors to detect R6G and detected it at a concentration of 10 pM [65]. High sensitivity could be attributed to low fluorescence, Raman background spectrum, and concentration effect. In order to enhance the SERS signal for identifying the analytes, new materials with Au/Ag combinations should be applied. The R6G detection is used to compare the performance of different SERS substrates. On the other hand, these aromatic dyes and their types are also important environmental contaminants, and their accurate detection is of great importance for the protection of the environment.

3.3.3 Detection of bacteria using paper-based SERS substrates

There is a great deal of concern about contaminants, food safety, and human health. Rapid and highly sensitive detection of pathogens in food and bacteria-induced disease has been of great interest for researchers. The SERS is extensively applied in detecting bacteria [66, 67] so that paper-based SERS biosensor with simple and high performance is also promising for future applications. Ankudze et al. [53] presented cotton fiber membrane-silver nanowires deposited on paper as a SERS biosensor (Figure 2) for effective detection of *Escherichia coli* extracted from urine. The silver nanowires were deposited onto the cotton fibers with APTES binder, and then the composite was hydraulically pressed into paper as a resistant and porous biosensor. The paper-based SERS biosensor could

detect up to 1×10^{-9} M of *Escherichia coli* in a urine sample.

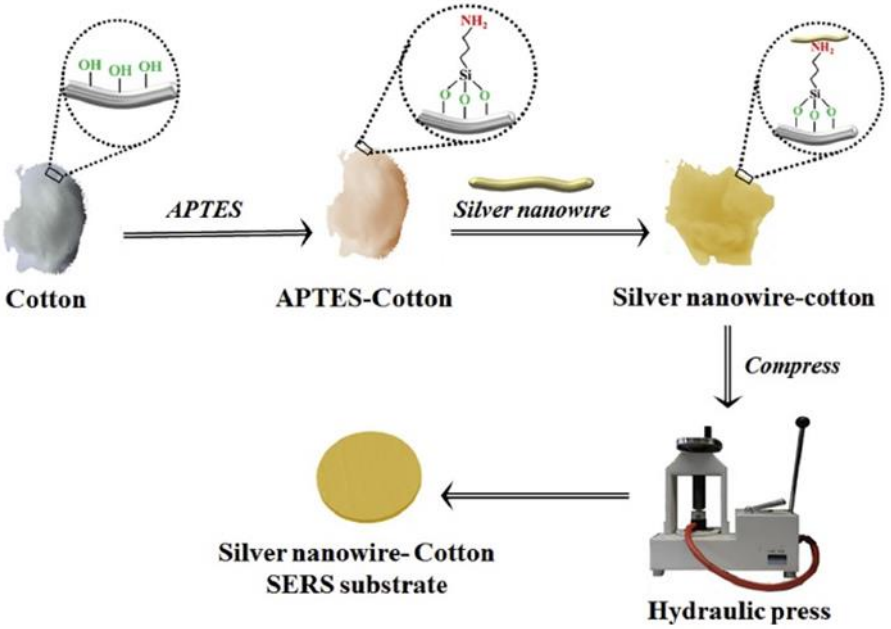

Figure 2. Schematic of cotton fiber membrane-silver nanowires deposited on paper as a SERS biosensor presented by Ankudze et al. [53].

Moreover, Lin et al. [56] developed a paper-based SERS biosensor using gold nanoparticles@mesoporous silica (AuNPs@MS) to detect *Staphylococcus aureus* in water (Figure 3). The gold nanoparticles deposited in silica were prepared using the substance calcination and then used in the form of a disk on paper and as a SERS biosensor to detect *Staphylococcus aureus* in water. The sensor operates in such a way that silanol groups (Si-OH) on the surface of AuNPs@MS establish a hydrogen bonding with the *Staphylococcus aureus* bacteria. Therefore, the *Staphylococcus aureus* bacteria in the water bind to gold nanoparticles, and as a result of the paper-based SERS biosensor, it detects the bacteria with a 900-fold enhancement in the SERS signal compared to the flat rigid substrate coated with Au/Cr (planar rigid Au/Cr-coated substrate). The effect of gold content on AuNPs@MS was also discussed, which showed that 16 wt% had the best performance.

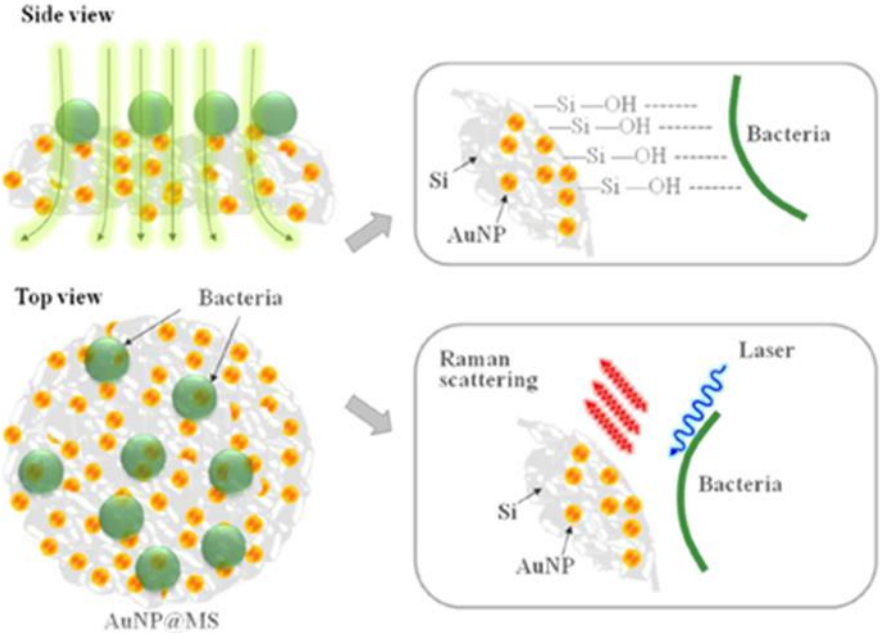

Figure 3. Schematic of a SERS substrate and bacteria [56].

Furthermore, Gao et al. [67] employed a paper-based SERS biosensor with a combination of specific aptamers to detect *Salmonella*. They used a label to bond a paper-based SERS biosensor to an aptamer to detect the SERS signal of *Salmonella*. The sensor could detect existing *Salmonella* cells with a volume of 1 mL and a number of 1000 CFU/mL for every 1 mL of sample untill a concentration of 4.7×10^3-1.4×10^7 CFU/mL.

3.3.4 Detection of pesticides using paper-based SERS substrates

Pesticides are extensively employed in modern agriculture; however, overuse of them also poses risks to human beings and the ecological system. Therefore, rapid and highly sensitive detection of pesticides in different samples is of great importance. The SERS has a particular advantage in the detection of pesticides due to its high sensitivity and specific fingerprint response. For instance, by applying extraction procedure and substrate functionalization, the detection of pesticides in real, complex matrix has been reported [68].

Paper-based SERS biosensors have high flexibility and are therefore suitable for detecting pesticides on different sample surfaces, some of which are discussed in this section. White et al. [20] used an ordinary syringe to deposit the silver colloidal solution on paper, succeeding in fabricating a paper-based SERS biosensor. They deposited the Malathion pesticide and detected a concentration of 61.5 ppb, which was 200 times better than conventional methods [20]. The fabrication of the paper-based SERS biosensor and its detection mechanism is straightforward and rapid. The assay performance is promising in terms of sensitivity, reproducibility, time efficiency, and cost-effectiveness [20]. Moreover, Fatexia et al. [69] developed a paper-based SERS biosensor using the active material Ag/LCP/PA to detect the Thiram pesticide in a standard solution and real samples. In order to fabricate the paper-based SERS biosensor, they used a paper substrate to create a network of liquid-crystal polymer fibers. Afterward, silver nanoparticles were deposited on it to fabricate a paper-based SERS biosensor, and then the target analyte was deposited on the fabricated sensor. In this way, they could detect the Thiram pesticide in river water and fruit juices. The limit of detection (LOD) for Thiram in the river water and fruit juices was 0.024 ppm and 5 ppm, respectively. The platform has great potential for environmental monitoring and biological detection, and the paper-based SERS biosensor shows excellent selectivity for the detection of Thiram.

4. Microfluidic paper-based SERS platforms

The microfluidic method creates a highly defined space in which fluid behavior, control, and manipulation can be addressed at microliter scales, and a variety of reactions may be handled quickly and correctly. Other obvious strengths include minimized sample consumption, quicker analysis and response, cost-effectiveness, and analysis time savings [70]. These technologies have been chosen for whole blood analysis because of the mentioned advantages [71]. When used independently, microfluidics and SERS have addressed some of the constraints of both technologies. Microfluidics allows for the precise manipulation of nanoparticles and their assemblies in channels related to microfluidic systems. The flow control and miniaturization in microfluidics also allow smaller substrates and lower sample volumes. Due to the low sample volumes and sensing areas, the adoption of a robust sensing technology such as SERS when handling microfluidics appears to be necessary [72].

Paper-based microfluidics can be regarded as a novel and alternative technique to fast analysis, in which the fabrication substrate is paper and microfluidic channels are formed on the surface. A paper-based microfluidic device has various benefits, including portability, user-friendliness, disposability, and cost-effectiveness. Moreover, it has the potential to be used to develop medical devices. Furthermore, due to the large surface-to-volume ratio, these devices are advantageous for some applications involving surface interactions, such as blood analysis [71]. Due to the wonderful advantages of paper-based microfluidic devices and given the merits of the combination of microfluidic and SERS, some valuable studies have been reported in the field of microfluidic paper-based SERS platforms in recent years, which are presented in this section.

Li et al. (2013) proposed a low-cost, quick, and efficient silver nanoparticles assay fabrication approach for SERS detection using a paper-microfluidic format (Figure 4) [70]. This approach did not need any special or costly equipment, and it eliminated time-consuming steps, allowed researchers to make high-performance SERS strips rapidly and affordably. They were able to accomplish high-sensitivity identification of 10^{-9} M R6G with excellent reproducibility using this approach, and the RSDs were less than 15% on different papers. More significantly, 1000 pieces of paper might be produced for less than $20. They believed that this simple spray method would be an excellent candidate for SERS applications, including environmental monitoring, food safety, and fast analysis and detection because of the paper's interesting properties such as cost efficiency, lighter weight, and straightforward identification of analytes.

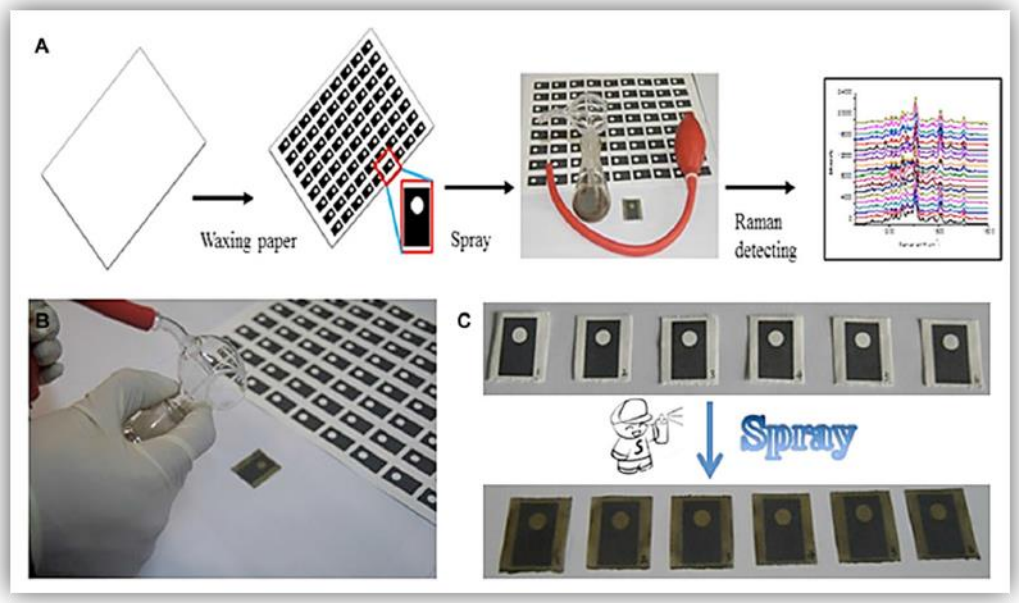

Figure 4. (A) Schematic diagram of the spraying method for fabricating paper-based microfluidic platforms for SERS applications. (B) Spraying silver nanoparticles onto the paper. (C) Same papers before and after spraying silver nanoparticles on its surface. Reprinted from Electrophoresis. 2013 Aug;34(15):2162-8, Li B, Zhang W, Chen L, Lin B. A fast and low-cost spray method for prototyping and depositing surface-enhanced Raman scattering arrays on microfluidic paper based device, Copyright 2013, with permission from John Wiley and Sons [70].

Torul et al. (2015) reported a paper membrane-based SERS system for identifying blood glucose levels, which used a nitrocellulose membrane as the substrate paper and a wax-printing process to create the microfluidic channel [71]. The 4-mercaptophenylboronic acid (4-MBA) and 1-decanethiol (1-DT) molecules were added to rod-shaped gold nanorod particles, which were then employed as embedded SERS probes for paper-based microfluidics. The SERS measurement area was created easily by dropping gold nanoparticles on membranes, and the blood sample was then dropped into the membrane's hydrophilic channel. Glucose molecules were transported via the channel into the SERS measurement area while blood cells and proteins were retained on the membrane. Moreover, the total analysis was completed in only 5 min. The proposed microfluidic paper-based SERS device was shown to be capable of detecting glucose in blood samples without the need for any pretreatment.

As indicated in Figure (5), Chen et al. (2016) explored a simple approach for on-site sulphite measurement in wine utilizing a gas-diffusion microfluidic paper-based analytical device (μPAD) integrated with SERS [73]. By sandwiching a ZnO-paper disc and combining gas preconcentration and gas-diffusion separation on a paper-based substrate, the cost-effective and disposable μPAD was created. The SERS signal at a shift of 620 cm^{-1} and SO_2 concentration demonstrated excellent linearity in the range of 5-300 μgmL^{-1} after extensive condition optimization. The LOD for sulphite was determined to be 2 μgmL^{-1}, with a linear correlation coefficient of 0.995. This proposed platform would allow extensive use for on-site sulphite monitoring due to its portability, low reagent consumption, and easy operation.

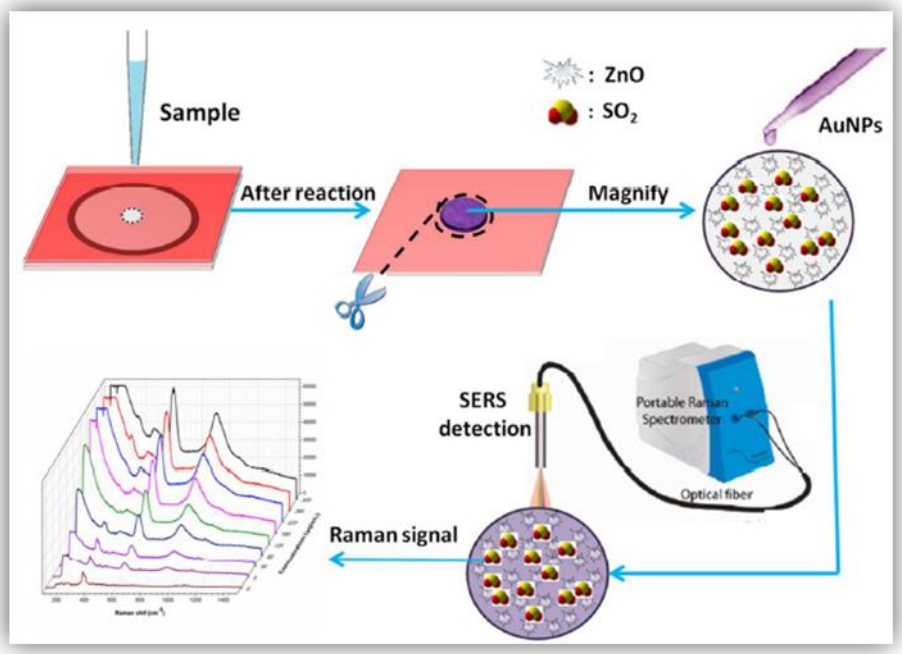

Figure 5. Illustration of µPAD-SERS for on-site detection of sulphite in wines. Reprinted from Analyst, Volume 141, Issue 19, Pages 5511-5519, Chen, Miao and Yang, Hua and Rong, Liya and Chen, Xiaoqing, A gas-diffusion microfluidic paper-based analytical device (µPAD) coupled with portable surface-enhanced Raman scattering (SERS): facile determination of sulphite in wines, Copyright 2016, with permission from The Royal Society of Chemistry [73].

With the advancement of industrial technology, new environmental issues have evolved due to the fabrication of many toxic compounds. Although various strategies have been developed to address these concerns, the outcomes have been unsatisfactory. Therefore, Lee et al. (2017) developed a label-free paper-based biosensing strip sensor for on-site analysis of wastewater components [74]. The chemically-vibrational reactions of the wastewater were fingerprinted using Raman spectroscopy. Moreover, the SERS effect was employed using gold nanoparticles to enhance the weak intensity of the Raman signals. A power-free successive ionic layer absorption and reaction (SILAR) approach was used to produce and distribute dense and uniform gold nanoparticles onto paper. For rhodamine 6G, the biosensing by SERS paper strip resulted in the sensitivity of 10^{-10} M and an enhancement factor of 2.8×10^{7}. It was shown that the SERS paper strip was sensitive to concentrations of 10^{-5} M and 10^{-9} M with correlation coefficients of 0.99 and 0.85 for pyrocatechol and 4-aminobenzoic acid, respectively. Thus, the reported gold nanoparticle-deposited SERS-encoded paper strip can be advantageous for on-site analysis wastewater. It might potentially be used for a variety of other applications.

For the identification of low concentrations of thiram, Zhu et al. (2017) used a paper-based microfluidic SERS platform [75]. The paper-based microfluidic device was printed by cutting a hydrophilic region from filter paper and pasting it onto sellotape. SERS probes were synthesized of Au@Ag nanoparticles (NPs) with a 30 nm Au core and a 7 nm Ag shell. The synthesized nanoparticles were dropped into one of the samples, which had paper-based microfluidics zones added to it, while the thiram solution was dropped into another. The solutions mixed together in the reaction chamber after flowing through their channels owing to capillary action. The peak at 1143 cm^{-1} was identified as an ideal peak for quantitative analysis of thiram solution because its intensity was highly sensitive in SERS experiments. Thiram had a low LOD of 1.0×10^{-9} mol/L, and RSD values from 10 random spots in the SERS measurement area were all less than 10%. Thiram recovery in adulterated tea samples ranged from 95% to 110%. These findings indicated that the proposed technology might be a feasible candidate for meeting crucial demands in food safety, environmental protection, and security.

Hariharan et al. (2019) demonstrated effective, specific, and sensitive catechol detection employing a simple, low-cost microfluidic paper-based substrate in combination with an enzymatic method [76]. Their method performed well in terms of linearity, precision, reproducibility, and LOD. Moreover, this sensor might be used to measure catechol levels in environmental samples in the future. The research might be expanded to include other o-diphenols, such as dopamine, a neurotransmitter, levels of which are related to mental health.

As shown in Figure (6), Lim et al. (2019) proposed a novel microfluidic SERS-based µPAD with multi reaction zones for simultaneous quantitative identification of various cardiac biomarkers, GPBB, CKMB, and cTnT, for early diagnosis and prognosis of acute myocardial infarction [77]. Three different Raman probes were created, then conjugated with the relevant detecting antibodies and employed as SERS nanotags to detect cardiac biomarkers. The LOD of their SERS-based µPAD was determined at 8, 10, and 1 pg mL^{-1} for GPBB, CKMB, and cTnT, respectively, under optimal conditions, which was significantly below the clinical threshold values. As a result, this proof-of-concept technology has much promise for ultrasensitive quantitative identification of multiplex cardiac biomarkers in blood, which might assist physicians in making better and immediate decisions.

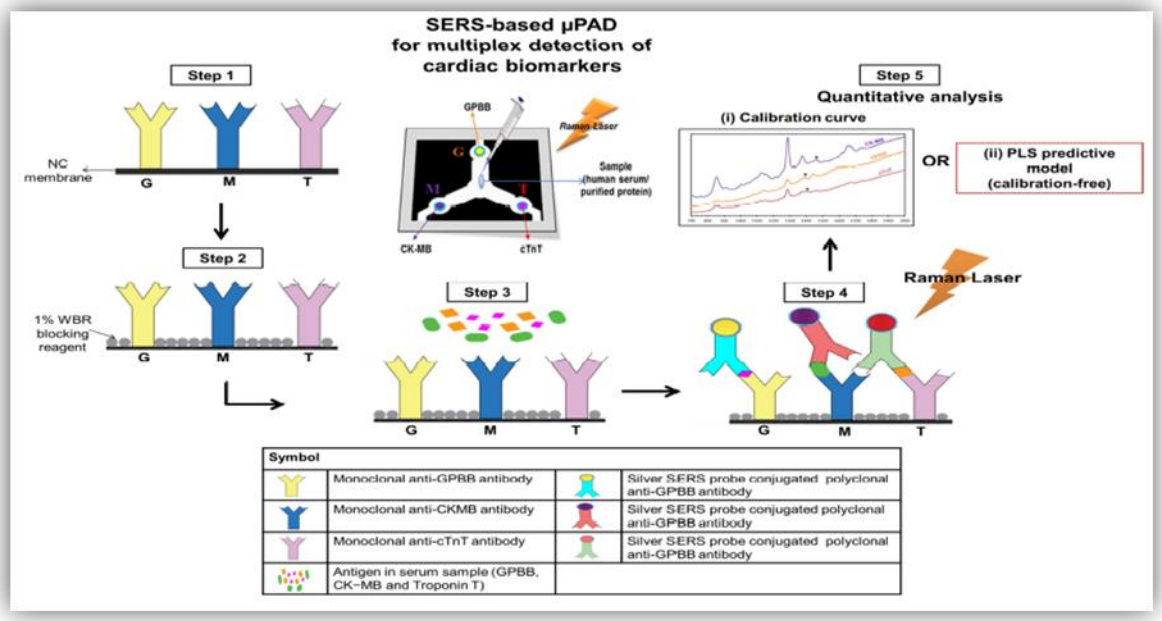

Figure 6. Schematic of multiplex SERS detection of cardiac biomarkers at respective reaction zone(s), G for GPBB, M for CK−MB, and T for cTnT on µPAD [77].

Mabbott et al. (2019) proposed a microfluidic paper-based diagnostic platform (Figure 7) that could detect miR-29a utilizing colorimetric and SERS analysis [78]. Through a combination of three-dimensional paper-based microfluidics, colorimetric detection, and SERS analysis, identification of miR-29a, a microRNA related to myocardial infarction, was accomplished at a level of pg μL^{-1}. Colorimetric output from samples containing miR-29a at various doses (18-360 pg μL^{-1}) demonstrated differentiation from the control sample. However, significant repeat variability showed that it could not be utilized to measure miR-29a levels. The SERS analysis, on the other hand, had better reproducibility at different concentrations, with a LOD of 47 pg μL^{-1}. The combination of the paper-based device and the two analysis techniques led to the development of a sensitive, reproducible, and simple point-of-care test, which can be used to diagnose a variety of diseases in the future.

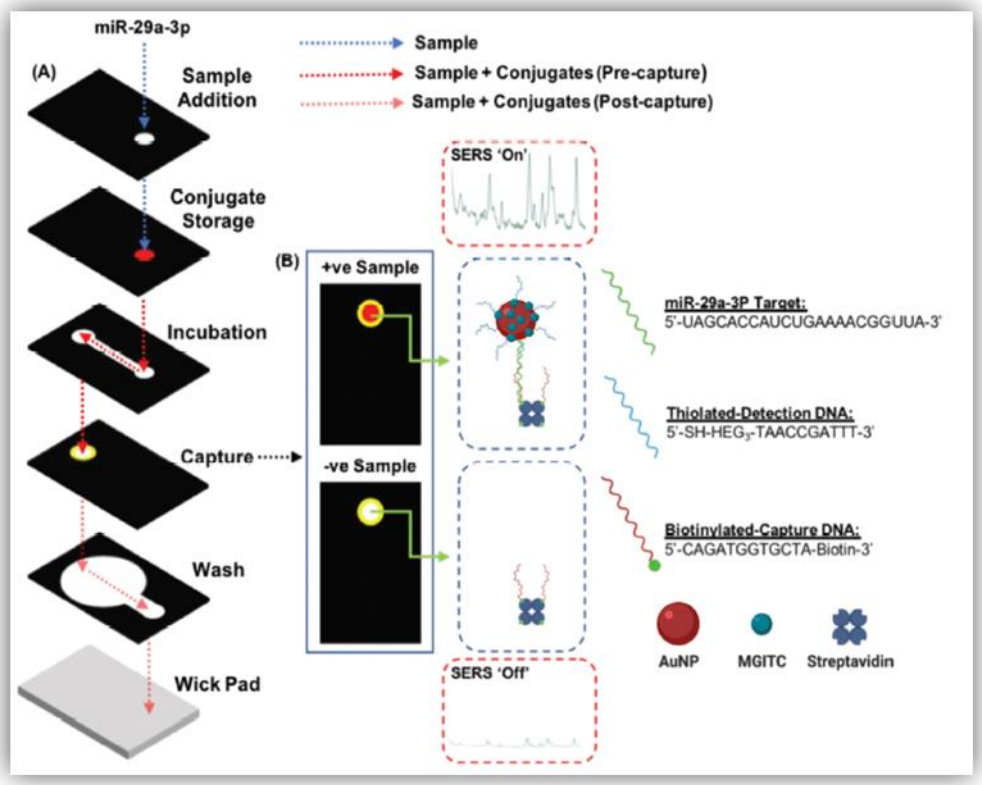

Figure 7. Schematic of a microfluidic paper-based diagnostic platform for the detection of miR-29a using colorimetric and SERS analysis [78].

The intrinsic reproducibility of microfluidics technology was applied in a study conducted by Teixeira et al. (2019) to fabricate self-assembled nanoparticle structures on a paper film [72]. In order to achieve ultra-sensitive detections, the paper-based SERS substrates were developed by assembling anisotropic particles, gold nanostars, and nanorods onto paper (Figure 8). The drying kinetics of the nanoparticles over the paper substrate was controlled using a polydimethylsiloxane PDMS-paper hybrid device. This approach allows for precise control of hot-spot formation while eliminating the coffee ring effect observed in previous nanoparticle assemblies. Furthermore, the assembly of the nanoparticles on the paper using this technique took less than 30 min, and numerous substrates could be created simultaneously. As a result, their approach for fabricating paper-based SERS substrates yielded excellent efficiency in terms of uniformity, surface coverage, duration, and portability, creating an opportunity to develop advanced nanotechnology-based diagnosis platforms.

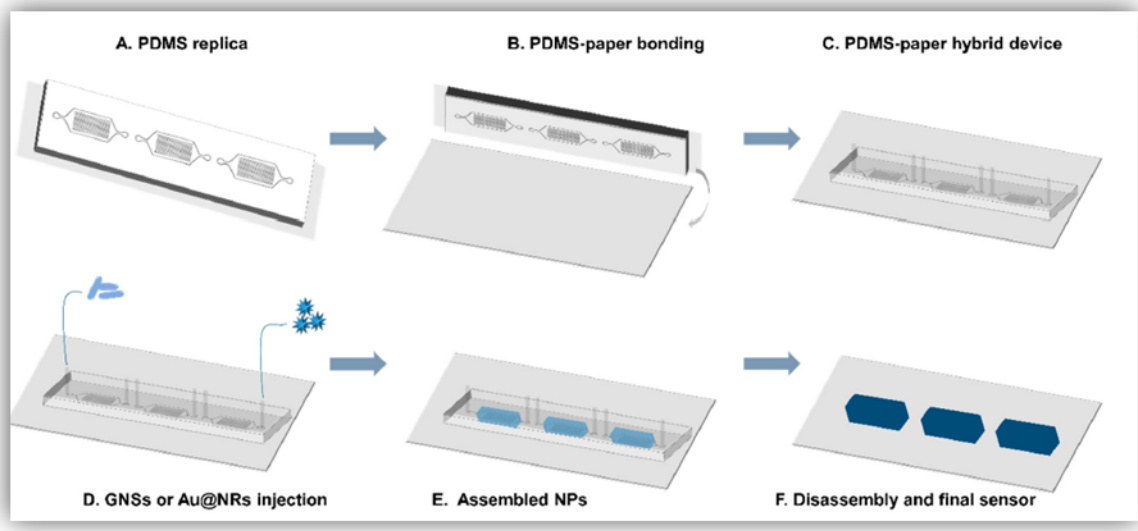

Figure 8. Schematic of the preparation of the hybrid PDMS-paper device (A–C) injection and self-assembly of the nanoparticles (D) Raman model molecule or lysed cells injection (E) final disassembly of the paper sensor for analysis (F) [72].

Some recent studies on microfluidic paper-based SERS platforms were reviewed in this section. Although these devices are greatly advantageous in various types of analyses, not many works have been conducted to develop and report these platforms. Accordingly, it can be considered an infant research field and needs to grow in future studies.

5. Conclusion and Perspectives

This review summarizes recent advances in the application of paper-based SERS platforms in the identification and detection of important biochemical species (particularly medical and biological). The fabrication of SERS-active substrates, due to simple fabrication mechanism, cost-effectiveness, and the ability to detect low concentrations of materials, is a new method for detecting toxic substances, narcotics, toxic industrial chemicals, and bio-analytes. As a result, SERS has excellent applications in the fields of security, medicine, and environmental monitoring. The development of new SERS substrate has always been a hot topic in SERS research. In addition to tracking the structure of components and assembling them, the development of flexible and versatile SERS substrate has been a new trend in recent years. Paper-based SERS substrates have several attractive features such as easy preparation, easy operation, low cost, high concentration, separation efficiency, and short detection time. Although paper-based SERS substrates have many advantages and tremendous potential in SERS analysis, their development still faces challenges, one of which is the accurate detection of target analytes with a much smaller sample volume. Moreover, the extensive applications of SERS biosensors demonstrate outstanding performance in bioassay and imaging, and applicable platforms have been developed. However, the application of paper-based SERS substrates in identifying biological materials still faces some limitations. Therefore, new paper-based substrates with multiple applications need to be studied. Recently, the development of substrates has become a new trend in SERS research. By combining Au/Ag with other materials, the resulting hybrid shows not only significant improvement in enhancement ability but also attractive features such as magnetic separation, reusability, and selective trapping. Moreover, paper-based SERS substrates have an inherent advantage in compression and separation, and their combination with new materials should provide more attractive properties for advanced analysis. Addressing these issues would accelerate the development of paper-based SERS substrates for practical analysis across a broader range. The rapid development of nanomaterials and nanotechnology may illuminate the future of paper-based SERS. Advanced techniques can be used to create micro or even nano papers with high efficiency in trapping or concentration. With the efforts of researchers and the development of modern nanotechnology, the paper-based SERS substrates are believed to be promising for a bright future as an alternative to many of the routine analytical techniques used in medical, biochemical, and biological analyzes. Furthermore, due to the need for low sample volumes, rapid analyses, low-cost systems, and time savings in detecting analytes, microfluidic paper-based SERS platforms have come to the scene in recent years. Despite the excellent capabilities of these advanced platforms, there is not extensive research regarding them; therefore, it seems to be essential to focus more on microfluidic paper-based SERS devices for the purpose of detecting a variety of materials, especially biomaterials and medical analytes.

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