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Rapid culture free Pathogen detection using SERS technique

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Surface-enhanced Raman scattering (SERS) spectroscopy has emerged as a versatile surface sensitive spectroscopic analytical tool on account of the gigantic augmentation of weak Raman signal and can assist appropriate detection of chemical and biological systems. SERS technique is so versatile that it can be employed from diverse applications ranging from plasmonics, sensing, catalysis to biomedical applications and diagnostics. This novel powerful technique has been utilized to detect pathogens including bacteria and viruses. In this paper, we have discussed the use of various SERS active substrates for the rapid identification of pathogens like viruses and bacteria. The pathogen detection by SERS technique represents a novel approach for rapid microbial diagnostics, where SERS can be directly applied on clinical sample rather than pure cultured one. We review comprehensively in a comparative manner the various studies involving the utilization of SERS technique to detect pathogens and provide a novel approach in terms of diagnostics applications. A few examples have been provided from the recent studies on the detection of pathogens by SERS technique from our research group © Anita Publications. All rights reserved.

Keywords: Bacteria, Spectroscopy, Raman scattering, Surface-enhanced Raman scattering, Silver nanoparticles, Biosynthesis, Pathogen detection.

1 Introduction

Raman scattering occurs as a consequence of interaction of electromagnetic radiation with matter, which results in the shift of frequency or wavelength of the incident radiation. After the development of strong, monochromatic, polarized and tunable lasers, the Raman spectroscopy has transformed into a highly sensitive technique to investigate the complicated molecular structure. On the other hand, the applications of conventional Raman spectroscopy are constrained as a result of the low scattering cross section of the Raman scattering process, which is ~ 12-14 orders of magnitude below fluorescence cross section for various biological and organic molecules [1-7]. Consequently, the unexpected high Raman signals obtained from pyridine molecules adsorbed on a rough silver electrode was demonstrated by Fleischmann and coworkers from the University of Southamption, United Kingdom in 1974 [8]. This outstanding discovery brought significant interest among researchers from various fields such as physics, chemistry, biology, mathematics and engineering. Surface-enhanced Raman scattering (SERS) takes place with the huge increase of the weak Raman scattering intensity by molecules in the vicinity of metallic nanostructured surfaces [5-8]. The SERS enhancement factor, which can be defined as the ratio between the Raman signals from a given number of molecules in the presence and in the absence of the metal nanostructure depends on the size as well as the shape of the nanostructures. SERS enhancement is generally around 10^6, but it may reach as high as 10^{10} at certain highly efficient sub-wavelength regions [5-8].

As evident from the literature, Raman spectroscopic technique has been applied for analyzing the composition of solids, liquids and gases. Therefore, this versatile technique can be utilized in diverse applications such as study of art and archeological materials (paintings, coins and sculptures), analysis of

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environmental pollutants, medical and biomedical applications, analysis of minerals and ores as well as detection of explosives [5,9,10,18].

Raman spectroscopy has broad medical and biomedical applications and can be used as a diagnostic, analytical tool in medical field as well as structural probe for various biological materials [10]. Recently, it has been applied both *ex-vivo* and *in- vivo* to deal with numerous biomedical phenomena; for example early detection and diagnosis of cancers [11], examining the outcome of different agents on the skin [12], determination of composition of herbal medicines to check the health and safety conformity of herbal products in the market [13], rapid detection of pathogenic microorganisms and analysis of tooth structure in dentistry [14]. Many studies have been reported in the literature on the oncological applications of Raman spectroscopy for the detection of malignant and non malignant tissues in different sites of body [15,16]. There are reports on the detection of breast, brain, cervical, gastrointestinal, lung, oral and skin cancers using Raman spectroscopy and its potential for *in- vivo* clinical implementation [15-17]. In dentistry, Raman spectroscopy has been used to determine the chemical structural properties of dentin and enamel in tooth [14]. However, most of the studies are still at the proof of concept stage. In addition, problems and issues yet need to be determined to bring this technology in hospital environment. However, low sensitivity problem of conventional Raman spectroscopy need to be solved for the clinical implementation of this versatile technique.

The discovery of SERS has solved the low sensitivity problem of conventional Raman spectroscopy and also perks up the surface sensitivity in general making the technique more appropriate. It also motivates the investigation of the interfacial processes relating enhanced optical scattering from adsorbates on metal surfaces [18].

SERS technique has different applications such as plasmonics, sensing, catalysis, medical and biomedical applications and diagnostics. This novel powerful technique has also been utilized to detect pathogens which include bacteria and viruses.

In this review article, we have shown the recent use of various SERS active substrates for the fast identification of pathogens like viruses and bacteria. We have reviewed in a comprehensive and comparative manner the range of studies involving the utilization of SERS technique to detect pathogens and provide a novel approach in terms of diagnostics application.

Due to space limitations, a complete review of all recent work on this new part of research is impossible. Nevertheless, a few examples including our own results had been abridged to demonstrate the recent development in SERS research in the detection of pathogens.

2 Historical background and gradual development of SERS

Raman spectroscopy, a spectroscopic technique is derived from molecular vibrations and inelastic scattering of monochromatic light, usually from a laser in the visible, near-infrared or near-ultraviolet region of electromagnetic spectra. This effect was discovered by famous Indian physicist Professor CV Raman in the year 1928 [19]. The weak Raman signal observed in conventional Raman spectroscopy can be explained by the low scattering cross-section (~10^{-30} cm^2 $molecule^{-1}$). Therefore, Raman spectroscopy provides low sensitivity in terms of signal and is the major cause for its inapplicability in practical fields for a long time [5-7].

Fleischmann and his group at the University of Southampton, United Kingdom performed Raman spectroscopic study with high intensity of signal by increasing the number of adsorbed molecules on a roughened metal electrode surface. In 1974, they reported very high quality Raman spectra of pyridine molecule adsorbed on electrochemically roughened Ag electrodes [8]. The authors attributed the enhancement in the Raman intensity to an increase in the surface area of the Ag electrode by the electrochemical roughening method.

Later, Jeanmaire and Van Duyne [20] from Northwestern University, USA, in 1977 first time recognized that surface area is not the key factor in the above phenomenon. Albrecht and Creighton [21] of University of Kent, UK, reported a comparable result in the same year. These two groups provided strong proofs to reveal that the strong surface Raman signal must be generated by a real enhancement of the Raman scattering efficiency (10^5 to 10^6 enhancement). The effect was later named as surface-enhanced Raman scattering and now, it is an universally accepted surface sensitive analytical technique. The exact mechanism of the enhancement effect of SERS is highly controversial as found in the literature. There are two major mechanisms accountable for the large enhancement effect of weak Raman signal obtained from pyridine molecules adsorbed on electrochemically roughened Ag surface. Jeanmaire and Van Duyne suggested an electromagnetic effect on the enhancement of Raman signal [20]. The electromagnetic theory is based on the excitation of localized surface plasmons. On the other hand, Albrecht and Creighton projected a theory based on the charge transfer effect of the adsorbed molecule on the enhancement efficiency [21]. This chemical enhancement theory relies on the charge transfer complex formation of the adsorbed molecule. However, it is very difficult to separate these two effects experimentally.

The most important highlight in the SERS research was the study of SERS spectra from single molecules (SM-SERS) by two research groups independently in 1997 [22,23]. The detection of single molecules using SERS technique and accomplishment of eventual limit of detection in any analytical detection was possible by combining other techniques, for example, fluorescence spectroscopy and scanning tunneling methods along with SERS techique.

Under appropriate environments, SERS enhancements of the order of 10^{14} can be obtained. It is important to mention here that special sites, occasionally referred to as “hot spots”, are accountable for the observed enhancement in SERS effect to a large extent. Therefore, current research work in SERS is focused on the controlled and reproducible fabrication of metallic nanostructures to form geometries like “hot spots” where the Raman probe molecules are properly located for large Raman enhancement. This will offer new information in novel research areas like plasmonics.

3 Applications of SERS

SERS is the most responsive analytical technique existing both to surface science and nanoscience, which can be applied along with other surface sensitive techniques to study various fundamental and applied areas for example corrosion, catalysis, advanced materials, diagnostics, and sensing.

Nie and Emory [22] carried out study on single-molecule SERS by employing SERS technique along with the transmission electron microscopy (TEM) and scanning tunneling microscopy (STM) techniques and observed Raman enhancement in the order of 10^{14} to 10^{15} for single rhodamine 6G (R6G) molecule adsorbed on selected Ag nanoparticles.

The surface-enhanced Raman scattering spectroscopy (SERS) can be employed for the detection of short live reaction intermediates such as radical and radical ions on the electrode surface and determination of the overall reaction mechanism. Tian and his group reported the first *in-situ* electrochemical SERS (EC-SERS) study on the electrochemical reduction of $PhCH_2Cl$ in acetonitrile (CH_3CN) on Ag electrode [24]. They detected benzyl radical anion as an intermediate and 3-phenylpropanenitrile as the major reaction product. The overall reaction mechanism involving the adsorption process of $PhCH_2Cl$ on the Ag surface and all other possible interactions including the solvent has been explained from the SERS study. The SERS results were further confirmed by theoretical quantum mechanical Density Functional Theory (DFT) calculations to recognize the reaction intermediate and products.

Mulvihill *et al* established that LB assemblies made of various polyhedral Ag nanocrystals can be used as SERS substrates for the high senstivity detection of arsenate and arsenite ions in aqueous solutions with a detection limit of 1 ppb [25]. The SERS substrate can be used as chemical sensor, which is both highly

reproducible as well as highly portable, and could be easily implemented in field detection. SERS technique can be further used in environmental analysis to study target molecules such as pesticides; herbicides; pharmaceutical chemicals in water; banned food dyes; chlorophenol derivatives and amino acids; chemical warfare species; explosives; and a variety of organic pollutants [26,27].

Immobilized metal nanoparticles in the form of SERS substrates can be used for biomedical diagnostics. For instance, the SERS substrate can be used as glucose sensor to detect glucose in human blood. Although, glucose is most commonly monitored by electrochemical-based sensors, a substitute protocol using SERS substrates fabricated by NSL technique has been employed to detect glucose in blood [28].

4 Applications of SERS in pathogen detection

SERS can be applied as a tool to distinguish pathogens such as bacteria and viruses [29,30]. Several research groups have reported the application of SERS-based assays for pathogen detection [29-32] ever since Efrima e*t al* first demonstrated the SERS spectrum of a bacterial cell surface [31,32]. Nevertheless, the majority of the SERS-based assays face a great deal of problems as a result of excessive fluctuations of SERS signals. These large fluctuations arise essentially from the non-homogeneity in the SERS-active substrates and due to incompatible binding between the bacterial cell surface and the SERS substrate. Metal colloids and nanostructures, which can be used as SERS active substrate, have poor biocompatibility. Therefore, it is necessary to develop new novel biocompatible substrates for Raman enhancement in case of biological molecules especially for rapid pathogen detection.

Liu *et al* used SERS-active Ag/AAO nanostructured system to monitor antibiotic induced chemical changes in bacterial cell wall [33]. The "chemical features" acquired from SERS spectrum of bacterial cell wall facilitates rapid identification of drug resistant bacteria within an hour. Furthermore, distinctive changes in the SERS profiles were observed in the drug-sensitive bacteria in the first phase (i.e., 1 hr) of antibiotic exposure, which could be used to differentiate them from the drug-resistant bacteria. The SERS based novel technique to detect pathogen was applied to a single bacterium. This rapid SERS detection of pathogens makes possible direct analysis of clinical specimen as an alternative to pure cultured specimen. It is important to mention here that conventional protocols for diagnosing bacterial infections based on the isolation of pure culture of the bacterium, followed by the determination of the identity of the isolate and an examination of the isolates responses to various antibiotics in terms of proliferation or viability. For such biological assays, an incubation period ranging from days to weeks or even months is required in order for the bacteria to grow to a density that can be handled by the available diagnostic tools. Over the past decade, several PCR-based methods have been developed for the identification of bacteria. Mass spectrometry is another substitute technique which has the potential for culture-free bacterial diagnostics. However, like the PCR approach, mass spectrometry depends on the available prior knowledge on the pathogens, which may or may not exist. Lastly yet importantly, neither of the PCR or mass spectrometry based approaches can be applied to live bacteria to monitor their responses to antibiotics or to conduct functional tests. However, SERS based spectroscopic method solve the limitation of PCR based methods. The newly developed SERS active substrates based on Ag/AAO system can be used for to observe the fine changes in the bacterial cell wall during the bacterium's different growth stages and of the bacterium's response to antibiotic treatment during early period of antibiotic exposure.

Recently, Ankamwar *et al* [34] fabricated a stable (more than three months) and homogeneous SERS active substrate from silver nanoparticles (Ag NPs) synthesized from leaf extract of *Neolamarckia cadamba* for the detection of two strains of bacteria, gram positive (*Staphylococcus aureus*, *S. aureus*) and gram negative (*Escherichia coli*, *E. coli*) bacteria. Figure 1 demonstrates the TEM image of the as-synthesized silver nanoparticles along with their UV-visible spectrum, SAED pattern and the resultant SERS spectra generated upon interaction with *S. aureus* and *E. coli*. These Ag NPs upon interaction with bacteria

can exhibit large Raman enhancement factor ((3 ± 0.20) × 10^7 and (5 ± 0.40) × 10^7 for *S. aureus* and *E. coli* bacteria, respectively) with almost zero fluctuations. The SERS substrate developed by them is nearly homogeneous with a relative standard deviation value of 6.32 calculated from 50 repeated measurements from various locations on the SERS substrate.

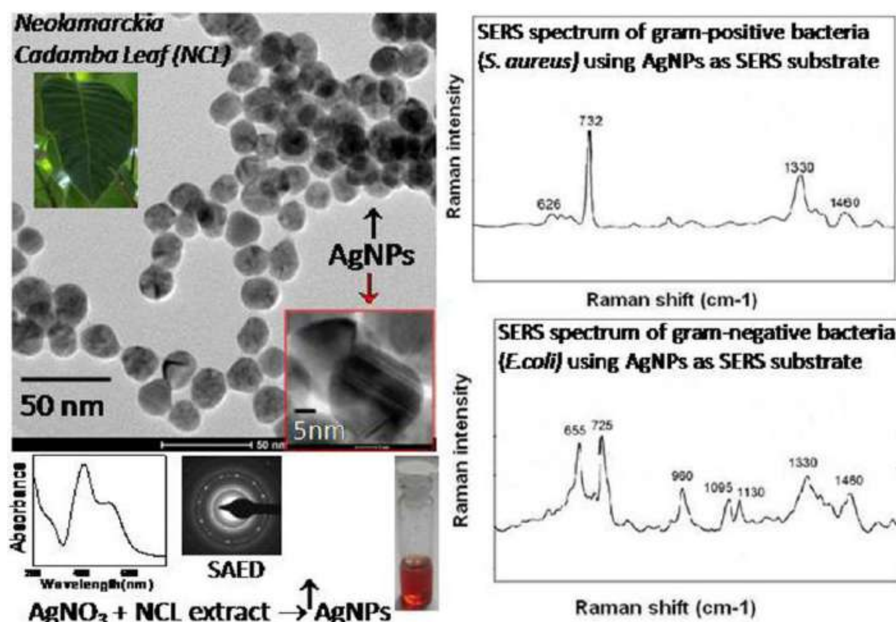

Fig 1. The TEM image of the biosynthesized silver nanoparticles along with the UV- vis spectrum, SAED pattern and the resultant SERS spectra generated upon interaction with *S. aureus* and *E. coli* bacteria. Reproduced with permission from Ankamwar B, Sur U K., Das P, *Anal Methods*, 2016; **8**: 2335-2340. Copyright @ Royal Society of Chemistry, Inc.

Besides this, the fabricated SERS substrate are extremely stable even after three months. Gram positive bacteria can be differentiated from Gram negative bacteria using this nearly homogeneous, stable SERS active substrate. The SERS data presented in this study is highly stable, uniform and reproducible, which shows the versatility of the biosynthesized SERS active substrate. This SERS active substrate is capable of detecting extremely low concentrations (10^3 CFU ml^{-1}) of *E. coli* and hence shows high sensitivity (see Fig 2). Figure 2 demonstrates the SERS calibration curve obtained with SERS intensity of the peak at 1330 cm^{-1} (C–N stretching mode) as a function of concentration of bacteria *E. coli*. The 1330 cm^{-1} peak became detectable at 10^3 CFU/ml of *E. coli* concentration. The SERS intensity increases with concentration of the bacterial solution, as it is exponentially correlated to the concentration of *E. coli* bacterial cells in the sample between 10^3 CFU/ml to 10^8 CFU/ml. Experiments were repeated five times with each bacterial concentration, and the standard errors of the mean for each concentration are also shown in Fig 2. The major intention of this SERS study using biosynthesized Ag nanoparticles was to develop a rapid fingerprinting method for the characterization of bacteria particularly *E. coli,* which is associated with urinary tract infection (UTI), a common disease among most people of all age groups in developed countries like India and China.

Biosynthesized Ag nanoparticles obtained from the plant extract of Reetha and Shikakai were utilized as SERS active substrate for rapid detection of harmful bacteria like *Mycobacterium tuberculosis*, which is recognized as drug-resistive to most of the common drugs available commercially [35]. Figure 3 shows the SERS spectrum of *Mycobacterium tuberculosis* on biosynthesized Ag nanoparticles. In the SERS spectrum, major peaks were observed at wavenumbers 437, 915, 1175 and 1390 cm^{-1}.

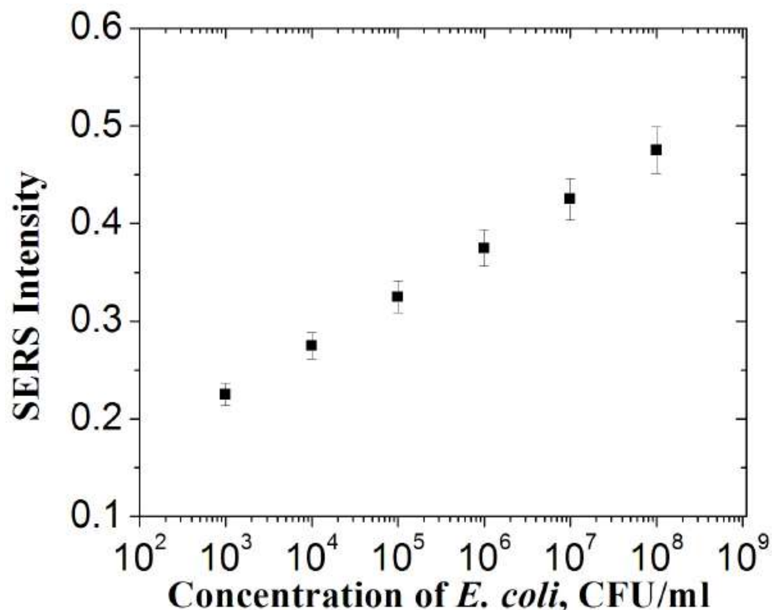

Fig 2. The SERS calibration curve obtained with SERS peak area or SERS intensity of the peak at 1330 cm^{-1} (C–N stretching mode) as a function of concentration of bacteria *E. coli*. Reproduced with permission from Ankamwar B, Sur U K, Das P, *Anal Methods*, 2016; **8**: 2335-2340. Copyright @ Royal Society of Chemistry, Inc.

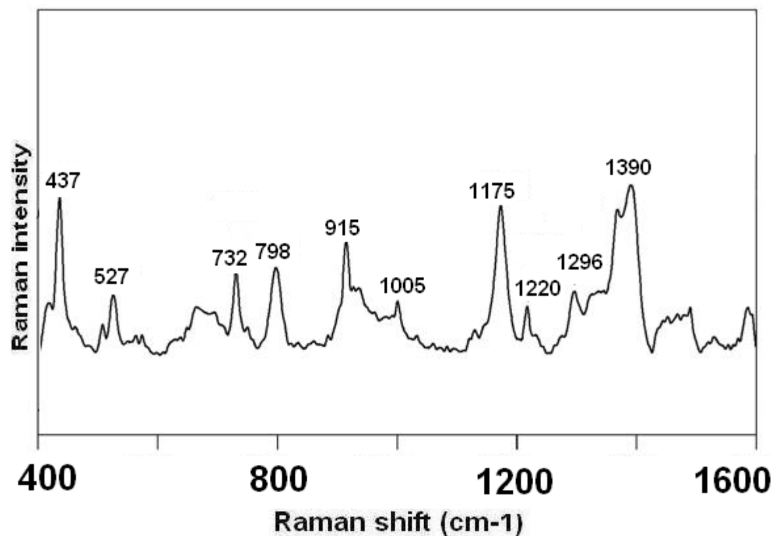

Fig 3. The SERS spectrum of bacteria *Mycobacterium tuberculosis* on biosynthesized Ag nanoparticles. Reproduced with permission from Sur U K, Ankamwar B, Karmakar S, Halder A, Das P, *Materials Today: Proceedings,*5 (2018) 2321-2329. . Copyright @ Elsevier Ltd.

The high quality SERS spectrum was observed by irradiating the whole bacterium via laser light as it interacts with the silver nanoparticles used as SERS substrate and it should principally reveal the information about molecular structure inside ten nanometers of the farthest bacterial cell wall [36]. The components as well as structural design of the bacterial cell wall are different in different bacteria and such

structural differences in cell wall should be clearly evident by SERS study. In this study, each SERS profile corresponds to the mean spectrum averaged from 10 samples after compilation of the SERS data within 10-20 second. Raman photon counts on the order tens of thousands unquestionably assign that these spectra are from the SERS process and not due to normal Raman scattering. The Raman enhancement factor for the biosynthesized Ag nanoparticles employed as the SERS substrate was anticipated from the calculation of the enhancement factor for 10 SERS samples and was found to be $(5 \pm 0.10)\times 10^9$. The SERS spectra recorded for *Mycobacterium tuberculosis* sample displayed unique characteristics which are dissimilar with respect to both peak position and intensity from the Gram-positive and the Gram-negative bacteria tested and reported earlier by the same group [34]. These characteristic SERS profiles might disclose the presence of mycolic acid and other components which are created in the cell wall of *Mycobacterium tuberculosis* bacteria. In comparison to the SERS data acquired from both Gram-positive and the Gram-negative bacteria, the 730 cm^{-1} peak is very feeble and the peak at 1330 cm^{-1} is completely absent in the SERS spectra of *Mycobacteria*. This distinctive feature may be due to the presence of long chain fatty acid in the outermost hydrophobic membrane of *Mycobacteria*, obstructing the peptidoglycan layer from impending towards the SERS substrate and thus withdrawing or completely removing the 730 and 1330 cm^{-1} peaks [37,38]. As a result, the compositions of the outermost membrane are comprising of biomolecules such as arabinogalactan, mycolic acids, lipids, which would make a major contribution in the observed extremely complex SERS spectra [38]. The SERS spectra of the *Mycobacterium* strains inactivated by heat treatment and formalin suspension inactivation illustrated nominal dissimilarities in contrast to the spectra of viable *Mycobacteria*. Consequently, it can be concluded that detection of *Mycobacteria* was possible without taking biosafety level 3 precautions during Raman measurements.

Numerous infectious diseases including lung tuberculosis (TB) in human being are caused by deadly bacteria *Mycobacterium tuberculosis* and it causes over two millions deaths yearly worldwide [39]. Therefore, the vigilant discovery of contributing pathogens is necessary for premature diagnosis, therapy, and control of this deadly disease. Several available conventional diagnostic techniques such as sputum smear microscopy, chest radiography and tuberculin skin testing are insensitive, laborious, prolonged and results are not accurate and frequently nonspecific [40,41]. Several rapid modern diagnostic protocols, which are commercially available in the market have been developed in order to improve the diagnostic accuracy for TB. PCR and other molecular amplification techniques are although both capable and prominent, none are more than additive to the diagnosis of TB due to variable sensitivities of the test results [42]. Additionally, these tests are specific for the detection of particular microorganisms and not applicable for diagnosing a wide spectrum of causative agents. Several rapid commercial techniques are now available for species identification of *M. tuberculosis* complex. However, these techniques are very expensive and limited to selected, frequently encountered species, as evident for available commercial techniques such as the reverse line blot assay, the Amplicor nucleic acid amplification test and the Gen-Probe Amplified Mycobacterium tuberculosis direct test [43]. However, the operation of 16S rRNA gene sequencing in routine practice at laboratories has a number of limitations, such as high cost, complexity, and the lack of peer-reviewed databases and clear explicit explanations. As a result of these constraints, there is enduring necessity for fast, simple option that can be readily applied to cultured bacteria from clinical material, which will facilitate the rapid detection of a wide spectrum of microorganisms. Although, thorough chemical information can be obtained from the SERS profile, it is very difficult to clearly understand it owing to the presence of any number of SERS-active vibrational modes in pathogens like bacteria.

Consequently, it is feasible to assign specific peaks indecisively from the prior biochemical information on the biochemistry of the pathogens to be investigated. In addition, the process of peak identification in SERS profile is more time-consuming due to the lack of proper database resource of SERS spectra. It is important to mention here that the aim of the SERS study using biosynthesized Ag nanoparticles was to develop SERS technique as a rapid fingerprinting diagnostic method for the qualitative and quantitative

characterization of bacteria of Mycobacterium groups with species particularly *Mycobacterium tuberculosis,* which is associated with deadly disease TB, a common disease among most people of all age groups in developed countries such as India, China, Thailand, other South Asian countries, African countries and Brazil. *Mycobacterium tuberculosis* is known to be drug-resistive to most of the common drugs. The SERS based rapid diagnostic technique will additionally assist in general understanding the role of various drugs including antibiotics and entire mechanism of the diagnosis of various diseases based on *Mycobacterium tuberculosis* bacteria and development of new drugs. It is expected that this study will generate breakthrough in overall understanding of microbial diagnostics.

5 Limitation of SERS technique in pathogen detection and future trend

It was demonstrated that the SERS technique can be useful for rapid detection of pathogens like bacteria and viruses based on the recently developed biosynthesized SERS-active substrate giving highly reproducible, stable and uniform Raman signal with large enhancement factor and almost zero fluctuation. One can potentially differentiate known or unknown pathogens rapidly within a few sec using the SERS spectra of bacterial cell wall as fingerprint. It is important to mention here that SERS based pathogen detection method is particularly helpful for the analysis of slow-growing bacteria, which normally may take weeks during laboratory tests.

On the other hand, the SERS based detection method of bacteria can not make a distinction of one strain from another within the same bacterial species. This is the most important drawback of the SERS based detection of bacteria. The SERS based technique for the detection of pathogens are inferior compared to genome sequencing or mass spectrometric based proteomics analysis in terms of the molecular level specificity.

However, quite a few original applications and functional tests can be carried out by utilizing the advantages of the new method's convenience, rapidity, stability and high sensitivity.

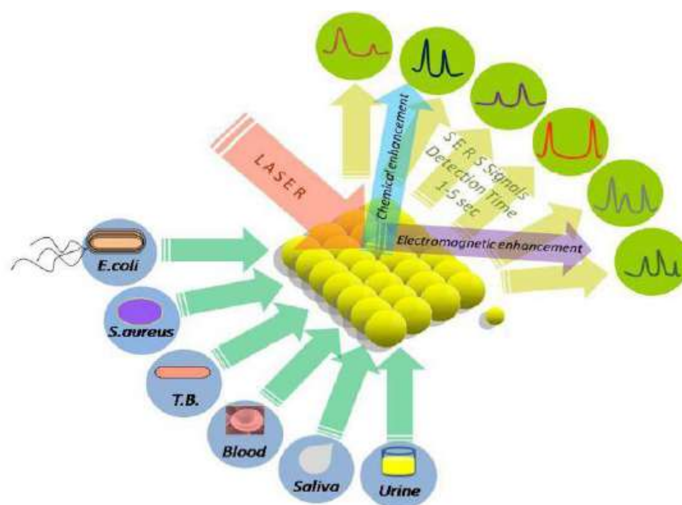

Fig 4. The schematic diagram explaining the SERS based pathogen detection along with its diagnostics applications from clinical samples. Photo courtesy B Ankamwar

Finally, the SERS technique is competent for evaluating the features of a single (live) bacterium and measuring the bacterium's antibiotic sensitivity. This novel platform provides an extraordinary prospect to study the physiological processes of an individual bacterium. This should allow SERS technique to be used to perform clinical microbial diagnostics directly on a clinical specimen without the need of bacterial pure

cultured samples, which are both time consuming and a little bit difficult to carry out. Clinical samples such as blood, urine, stool, saliva, sputum which are directly collected from the infected persons and patients in the hospitals and can be subsequently utilized to carry out SERS base detection technique of pathogens. Figure 4 shows the schematic diagram explaining the SERS based pathogen detection along with its diagnostics applications from clinical samples.

It is expected that several new and unknown diseases like Swine Flu, Avian flu, Japanese Encephalitis, Ebola, Zika and Dengue which are very difficult to detect within a short period of time, can be qualitatively and quantitatively detected using the newly developed SERS based protocols. The detection of these harmful pathogens will facilitate the development of vaccines to overcome or control the diseases among the masses in the form of epidemic. In addition to this, prior and extensive knowledge of the pathogens and its rapid extensive characterization is essential for the development of new drugs. It is important to mention here that diseases like Swine Flu, Avian flu, Japanese Encephalitis and Dengue have emerged as lifethreatening and harmful in different parts of the world in recent years. It is essential to develop new diagnostic tools and sensing devices to control these new diseases.

6 Conclusions

Surface-enhanced Raman scattering (SERS) deals with the increase of the weak Raman scattering intensity by molecules in the presence of nanostructured metallic surfaces especially gold and silver nanoparticles. It has developed into a versatile spectroscopic and analytical technique due to the rapid progress of nanoscience and nanotechnology for extremely sensitive and selective recognition of chemical and biological systems. The augmentation of Raman signal is known to initiate from the strong optical intensity localized within 10 nm from the surface of metallic nanostructures. Using SERS, the chemical features within this range from the surface of the SERS-active substrate can be detected and analyzed in an extremely sensitive manner.

The sensitive and stable SERS profiles along with the “chemical features” obtained from SERS spectrum of bacterial cell wall facilitates speedy recognition of pathogens like bacteria and viruses within a very short time scale. Rapid and accurate diagnosis for pathogens and their antibiotic susceptibility is critical for controlling bacterial infections. Conventional methods for determining bacterium’s sensitivity to antibiotic depend mostly on measuring the change of microbial proliferation in response to the drug. Such ‘‘biological assay’’ inevitably takes time, ranging from days to weeks.

In this review article, we have demonstrated the recent use of various SERS active substrates for the rapid identification of pathogens like viruses and bacteria. It is expected that this kind of study will produce breakthrough in overall understanding of microbial diagnostics.

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References

1. Van Duyne R P, In Chemical and Biological Applications of Lasers, (ed) C B Moore, (Academic Press, New York), 1979.
2. Chang R K, Furtak T E, In Surface Enhanced Raman Scattering, (Plenum Press, New York), 1982.

3. Aroca R, In Surface enhanced Vibrational Spectroscopy, (John Wiley and Sons, United Kingdom), 2006.
4. Tian Z Q, Ren B, In Encyclopedia of Electrochemistry, (ed) P Unwin, A J Bard, M Stratmann, (Wiley-VCH, Weinheim), 2003, p. 572.
5. Sur U K, *Resonance*, 15(2010)154-164.
6. Sur U K, Chowdhury J, *Curr Sci*, 105(2013)923-939.
7. Sur U K, *Adv Nano Res*, 1(2013)111.
8. Fleischmann M, Hendra P J, McQuillan A J, *Chem Phys Lett*, 26(1974)163-166.
9. Withnall R, Shadi I, Chowdhry B, In Raman Spectroscopy in Archaeology and Art History, (eds) H G M Edwards, J M Chalmers, (RSC Analytical Spectroscopy monographs, Royal Society of Chemistry, Cambridge), 2005.
10. Ozaki Y, *Appl Spectrosc Rev*, 24(1988)259-312.
11. Haka A S, Volynskaya Z, Gardecki J A, Nazemi J, Lyons J, Hicks D, Fitzmaurice M, Dasari R R, Crowe J P, Feld M S, *Cancer Res*, 66 (2006)3317-3322.
12. Choo-Smith L-P, Edwards HGM, Endtz H P, Kros J M, Heule F, Barr H, Robinson J S (Jr), Bruining H A, Puppels G J, *Biopolymers*, 67(2002)1-9.
13. Huang C C, *Appl Spectrosc Rev,* 51(2016)1-11.
14. Ramakrishnaiah R, ur Rehman G, Basavarajappa S, Al Khuraif A A, Durgesh B H, Khan A S, ur Rehman I, *Appl Spectrosc Rev,* 50(2015)332-350.
15. Haka A S, Shafer-Peltier K E, Fitzmaurice M, Crowe J, Dasari R R, Feld M S, *Proc Natl Acad Sci, USA*, 102 (2005)12371-12376.
16. Nijssen A, Koljenovic S, Schut T C B, Caspers P J, Puppels G J, *J Biophotonics,* 2(2009)29-36.
17. Santos I P, Barroso E M, Schut TCB, Caspers P J, van Lanschot C G F, Choi D-H, van der Kamp M F, Smits R W H, van Doorn R, Verdijk R M, Hegt V N, von der Thüsen J H, van Deurzen C H M, Koppert L B, van Leenders G J L H, Ewing-Graham P C, van Doorn H C, Dirven C M F, Busstra M B, Hardillo J, Sewnaik A, ten Hove I, Mast H, Monserez D A, Meeuwis C, Nijsten T, Wolvius E B, de Jong R J B, Puppels G J, Koljenović S, *Analyst*, 142 (2017)3025-3047.
18. Cooney R P, Mahoney M R, McQuillan A J, *In* Advances of Infrared and Raman Spectroscopy, (eds) R J H Clark, R E Hester, (Heyden, London), 1982, p. 188.
19. (a) Raman C V, Krishnan K S, *Nature*, 121(1928)501-502.
    (b) Singh R, Rastogi V K, *Asian J Phys*, 27(2018)93-101.
20. Jeanmaire D L, Van Duyne R P, *J Electroanal Chem*, 84(1977)1-20.
21. Albrecht M G, Creighton J A, *J Am Chem Soc*, 99(1977)5215-5217.
22. Nie S, Emory S R, *Science*, 275(1997)1102-1106.
23. Kneipp K, Wang Y, Kneipp H, Perelman L T, Itzkan I, Dasari R R, Field M S, *Phys Rev Lett*, 78 (1997)1667-1670; doi.org/10.1103/PhysRevLett.78.1667
24. Wang A, Huang Y F, Sur U K, Wu D Y, Ren B, Rondinini S, Amatore C, Tian Z Q, *J Am Chem Soc*, 13(2010) 9534-9536.
25. Mulvihill M, Tao A, Benjauthrit K, Arnold J, Yang P, *Angew Chem Int Ed*, 47(2008) 6456-6460.
26. Liu S Q, Tang Z Y, *J Mater Chem*, 20(2010)24-35.
27. Fan M, Andrade G F S, Brolo A G, *Analytica Chimica Acta*, 693(2011)7.
28. Shafer-Peltier K E, Haynes C L, Glucksberg M R, Van Duyne R P, *J Am Chem Soc*, 125(2003)588-593.
29. Jarvis R M, Goodacre R, *Anal Chem*, 76(2004)40-47.
30. Sengupta A, Laucks M L, Davis E J, *Appl Spectrosc*, 59(2005)1016-1023.
31. Efrima S, Bronk B V, *J Phys Chem B*, 102(1998)5947-5950.
32. Efrima S, Zeiri L, *J Raman Spectrosc,* 40(2009)277-288.
33. Liu T T, Lin Y H, Hung C S, Liu T J, Chen Y, Huang Y C, Tsai T H, Wang H H, Wang D W, Wang J K, Wang Y L, Lin C H, *PLoS ONE,* 4(2009)1; doi.org/10.1371/journal.

34. Ankamwar B, Sur U K, Das P, *Anal Methods*, 8(2016)2335-2340.
35. Sur U K, Ankamwar B, Karmakar S, Halder A, Das P, *Materials Today: Proceedings,* 5(2018)2321.
36. Kahraman M, Yazici M M, Sahin F, Bayrak O F, Culha M, *Appl Spectrosc*, 61(2007)479-485.
37. Hoffmann C, Leis A, Niederweis M, Plitzko J M, Engelhardt H, *Proc Nat Acad Sci*, 105 (2008) 3963; doi.org/10.1073/pnas.0709530105
38. Buijtels P C A M, Willemse-Erix H F M, Petit P L C, Endtz H P, Puppels G J, Verbrugh H A, van Belkum A, van Soolingen D, Maquelin K, *J Clin Microbiol*, 46(2008)961-965.
39. WHO report 2006, (2006) World Health Organization, Geneva, Switzerland.
40. Cantanzaro A, Davidson B L, Fuziwara P L, Goldberger M J, Gordin F, Salfinger M, Sbarbaro J, Schluger N W, Sierra M F, Woods G, American Thoracic Society Workshop, *Am J Respir Crit Care Med*, 155(1997)1804-1814.
41. Buijtels P C, Petit P L, Verbrugh H A,van Belkum A,van Soolingen D, *J Clin Microbiol*, 43(2005)6020-6026.
42. Nahid P, Pai M, Hopewell P C, *Proc Am Thorac Soc*, 3(2006)103-110.
43. Kirschner P, Bottger E C, *Methods Mol Biol*, 101(1998)349-361.

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