The Pharma Innovation Journal 2021; SP-10(10): 1195-1202

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; SP-10(10): 1195-1202

www.thepharmajournal.com
Received: 22-07-2021
Accepted: 05-09-2021

Akashamrut M Patel
Food Safety and Testing Department, College of Food Processing Technology & Bioenergy, Anand Agricultural University, Anand, Gujarat, India

Bhavesh H Joshi
Food Quality Assurance Department, College of Food Processing Technology & Bioenergy, Anand Agricultural University, Anand, Gujarat, India

Rashmin M Dhingani
Food Quality Assurance Department, College of Food Processing Technology & Bioenergy, Anand Agricultural University, Anand, Gujarat, India

Corresponding Author
Akashamrut M Patel
Food Safety and Testing Department, College of Food Processing Technology & Bioenergy, Anand Agricultural University, Anand, Gujarat, India

Surface enhanced Raman spectroscopy for detection of pathogens: A review

Akashamrut M Patel, Bhavesh H Joshi and Rashmin M Dhingani

Abstract
Presence of food pathogens in any food can have serious consequences and thus their rapid and accurate detection is of enormous economic importance. Surface Enhanced Raman Spectroscopy (SERS) is promising for food pathogen detection as it promises rapid detection and quantification and thus reviewed in present study for the same. It was found in review that due to ever decreasing costs of electronics is making handled SERS devices practical and thus filed detection of pathogens using SERS has good future. It was also discovered that SERS has its own set of limitations particularly when it comes to quantification instead of just detection i.e. requirement of bench top equipment in addition to cost and availability of surface enhance Raman active substrates.

Keywords: Food pathogen detection, surface enhanced Raman, review

Introduction

History

Sir C. V. Raman discovered Raman effect in 1928 and won noble price in 1930 for it. After around 45 years SERS from pyridine adsorbed on electrochemically roughened silver was first observed by Martin Fleischmann, Patrick J. Hendra and A. James McQuillan at University of Southampton, UK in 1973. Since then filed is in continuous research and development. Modern SERS technique can be classified as optical biosensor when used for pathogen detection.

Basic Principle

Raman spectroscopy depends on Raman scattering phenomenon. Normally light is scattered at same frequency as that of incident light but few photons experience either bit of red or blue shift. If we filter out incident light we can observe this Raman scattering. Naturally Raman scattering is weak and thus enhancing mechanisms are required. It is observed that Raman scattering is much more if sample is near the appropriate surface and this new technique was named surface enhanced Raman. In reality surface may be surface of particle freely moving in solution i.e. immobile surface is not necessary. The enhancement factor can be as much as 10E10 to 10E11, which means the technique may detect single molecules. Enhancement is ascribed to two mechanisms i.e. electromagnetic and chemical.

Raman Reporters

If substance is not Raman active reporter molecules are required i.e. 4-aminothiophenol also called 4-ATP, 4-Nitrothiophenol also known as 4 NTP and similar. Note that thiol group if present in reporter it can be used to connect it with gold or silver and it is present for example in 4ATP and 4NTP. Other reporters: 3-Amino-1,2,4-triazole-5-thiol = ATT, 4-mercaptobenzoic acid = 4-MBA, Rhodamine 6G = Rh6G, 4-mercaptopyridine = 4-MPY, 5,5-dithiobis-2-nitrobenzoic acid = DTNB

Fabrication of Particles or Surfaces for Enhancement

Nano particles can be formed by agglomeration by various mechanisms. Particles can be attached to metals or can form agglomerates for enhanced hot spots. Lithography is useful in itching precise shapes on hard surfaces and this surfaces can be used as templates to form nano particles or one can just coat surface with metal [1]. It is possible to make particles in various shapes and sizes [2, 3].

- SERS signal faces problem of reproducibility as it depends on binding geometry which may be different every time but this can be averaged out.

Fabrication of very good SERS substrate is difficult. Achieving quantitative analysis in highly diluted solutions is also difficult.

- There are nonmetal options for SERS i.e. paper, carbon, silicon (which is half metal).
- NIR radiation is absorbed less than visible light and thus can be used to examine deeper inside samples provided that NIR active Raman substrate is present there to generate signal. Cyanine dyes and GNPs are commonly used with NIR Raman as enhancers.
- Multilayer nanoparticles provide more enhancement compared to monolayer i.e. both material provide enhancement.
- Aggregated nanostructures are the earliest SER substrates. Only 1% of the surface of the aggregates is actually do enhancement due to presence of hot spots and thus this indicates chance to improvement. Lithography can be used to precisely control nanostructures but it can also generate reproducible but random nanostructures.
- Self-assembled or designed nanostructures give uniform and regular surface and much better enhancement compared to random structures.
- Instead of using smooth round nanoparticles, nanoparticles with spines give better signal enhancement for example rod or start shaped nanoparticles.
- Gold nanostars are a class of novel spiky NPs with a spherical core and many outward branching sharp tips. Nano flowers and nano pyramids are also reported.
- Plasmonic nano-satellites type structures composed of a metallic core and multiple satellite NPs around the core, demonstrate intense plasmonic coupling between high density satellite NPs. The strong plasmonic coupling remarkably increases the EM field intensity between the core and satellite NPs, resulting in enormous enhancement in hotspots. Aptamers are commonly used to connect satellites to main particle.
- Graphene oxide layer enhances Raman scattering by charge transfer with analyte but enhancement by graphene itself is not in useful wavelength range and is weak in general. Main action of graphene is by improved adsorption for compatible analytes and improvement in enhancement occurred by other SERS substrates by increasing coupling hot spots.
- Carbon nanotubes can also act as SERS substrate.
- Silicon materials incorporated with noble metal nanoparticles can improve SERS due to coupling and can increasing hot spots. Nanoparticle coated silicon nano wires, nano cones, nano spikes etc. are successful among which silicone nanowires are most common. Silicone can also be used as coating material.
- Alloy nano particles are also used. For example stability of silver gold alloy nanoparticles is better than silver nanoparticles because gold is more inert than silver. Ag/Au nanoparticles also exhibit better stability.
- Magnetic nanoparticles give unique handling convenience i.e. $Fe_3O_4/SiO_2/Ag$ or $Fe_3O_4/SiO_2/Au$ core shell NP Coating nanoparticles or Fe_3O_4/Au core–shell NP. Can be used to detect bacteria.
- 3D nanostructures offer more hot spots compared to 2D geometries. Also more analyte is captured near hot spots. 3D nano structures can be prepared by taking suitable substances and then evaporating solvent.

In general, intensive SERS signals are generated when the target analytes have high affinity to SERS substrate. Highly efficient adsorption mechanism is quite helpful. This can be achieved by adding molecules which connect analyte to metal surface by covalent bond or by weak interaction i.e. hydrophilic, phobic, ionic etc. or in other words antibody like mechanisms. Note that adsorption should be specific but non-specific adsorption will also help as Raman signal itself is specific to particular bonds.

- Micro fluidic platforms can help in many situations for example enhancement by regular pattern itched on surface. Pathogen detection can happen on microfluidics by combining immunological detection with Raman method. Paper based tests are also developed which work alike to lateral flow assay but with detection using SER. Such paper can be prepared by printing or other deposition methods [4].

In general, SERS active nanostructures can be divided into two types of substrates: colloidal substrates and solid surface based substrates. Using the colloid substrates is the most direct way to attain enhanced Raman signal, although in solution-based aqueous systems, it is not easy to control the assembly of metallic nanoparticles and to evaluate the exact location of the target analytes. In contrast, solid surface-based substrates can have well control on the development of hot spots and the exact location of target analytes, consequently displaying an improved reproducibility and enhancement. A major challenge for employing colloids is their weak signal reproducibility. If these colloidal substrates are used in dried form on a solid surface, the nanoparticles (NPs) incline to be casually accumulated and, consequently, the signals are highly variable from spot to spot. Control of distributed NPs into a uniform style, such as a dendritic pattern, or incorporation of colloids into a sol-gel can greatly enhance their reproducibility.

Industrial applications need highly reproducible, strongly enhanced, and long-term stable SERS substrates. Therefore, due to the growing popularity of SERS for trace level detection, different commercial solid surface-based SERS substrates have also been developed. Although these substrates are comparatively costly, they provide the benefits of comparatively reliable outcomes and fast and easy sample preparation by just pouring the target molecule solution on the surface of the substrate.

Moreover, there have been struggles to generate flexible, adhesive, nanostructure arrays that are SERS-active. They can be easily applied on irregular surfaces to identify chemical agents in situ and in real-time. Great efforts have thus been made to develop flexible substrate materials such as cotton, polymer, and paper i.e., paper swab with noble metallic NPs offers the convenience and efficiency for SERS detection by swabbing. PDMS based soft lithography can be used to make SERS surfaces. SERS tapes have been employed to directly identify, detect and extract pesticide residues on fruit and vegetables through a simple ‘paste and peel off’ sampling method. Besides the flexible tapes, inkjet printing of Ag NPs on plastic polyethylene terephthalate (PET) was also available to generate flexible and active SERS substrates.

It has been proved that adhesive and flexible SERS substrates are a promising technique for practical application in on-site trace analysis for the food industry. However, the solid surface based substrates are comparatively difficult to be fabricated, but there is no fear of aggregation and they retain better reproducibility, stability and sensitivity [5].

Optimization of SERS substrate using Simulations

Mathematic calculations can be used to design and optimize SER surfaces and particles. The mathematical technique is Finite-Difference Time-Domain (FDTD) and used to create computer simulations of electron behavior. Such designed substrates were used in various detections and determinations including viruses. Most of nanoparticles used in various studies were multi-layer [6].

Uses other Than Pathogen Testing

SERS can be used for detection and determination at molecular level. It is also used for adulteration detection in food products [7]. Also used for biosensors, detect warfare agents, single molecules sensing [8].

Detection of Pathogens

Two approaches are sued commonly. In labeled detection antibody as well as Raman reporter are generally present. In label free approach microbes directly attach on SERS surface and give signal.

SERS detection of spore-forming bacteria, such as *Bacillus*, commonly depends on the typical biomarker such as calcium dipicolinate (CaDPA) or dipicolinic acid (DPA) from bacterial spores. In most cases, SERS spectra of whole bacteria cell without spores were found to originate from the interior or exterior of bacterial cell. Generally exterior of bacterial cell wall determines signal. This happens because SERS signal is strongest near the surface. For specific detection antibody mechanism can be used.

All the bacteria types have similar peaks in the given spectral region 520 and 1050 cm^{-1} with some difference in frequency for certain peaks. The SERS profile of bacteria offer signature of molecular structures, cellular compositions, and physiological states, which can be used to discriminate different bacteria species or feature live and dead bacteria. Different SERS spectra of dead bacteria from live ones may contribute to the rupture of outer layer of dead bacterial cell wall.

SERS peaks helps to characterize the complex biomatrix in multispecies biofilms and evaluate the chemical components in the matrix of biofilm at different growth phases. For instance, peaks at 918, 1167, 1223, and 1333 cm^{-1} of *P. aeruginosa* biofilms assigned to carbohydrates and proteins were too weak in early and mid-stage, but increased substantially in the late stage.

Vancomycin antibiotic, can strongly bind bacterial cells via hydrogen bonds between peptidoglycan on the bacteria cell wall and carbonyl and amine groups of vancomycin. vancomycin can pull portion of cell wall closer to hot junctions of SERS substrate, leading to a very large and stable enhancement of SERS signal. Signal was better than control for both G+ and G- bacteria and noise is reduced due to selective binding. The SERS substrate was nano structured metal surface.

The popular label-free SERS based bacterial analysis usually employed Au NPs or Ag NPs colloidal or particle aggregates. Aggregation is induced after or upon addition of bacteria and aggregates forms directly on bacterial surfaces. Though simply mixing strategy makes detection cheaper, easier and faster, the generated mixture is not always homogenous which leads to random distribution of NPs on surface of pathogen cells then irreproducible SERS spectra. The main goal for NPs based SERS method is to make the NPs come into contact with the bacteria surface as many points and close as possible. Ag NPs has been widely used as an active-SERS substrate for bacteria detection due to their superior SERS enhancement. Several strategies such as co-incubation, electrostatic interaction, in-situ synthesis of NPs were adopted to achieve such objective.

Electrostatic attraction force strategy adopted to fabricate NPs on bacteria cell wall in an efficient and precise way attracts much attention. This happens because the cell wall of G- or G+ bacteria is negatively charged due to the presence of outer membrane lipopolysaccharides or teichoic acid, respectively. The enhancement of Raman signal of bacteria using this strategy is much higher than that in the case of a simply mixed colloid-bacterial suspension (30-fold). The LOD obtained was 2.5×102 cell/ml. Zeta potential affects electro statics interactions and thus signal strength can be controlled by controlling pH.

SERS based micro arrays are successfully used to detect pathogens. No reporter molecules were used but antibodies were used. Synthesizing nano particles on bacteria after capturing them on micro array gave 10 fold improvement in detection i.e. 10E2 CFU/g. Common antibodies used in micro array cannot provide strain level specificity is problem. Micro array sensitivity is high but dynamic range is limited i.e. either we count well in positive or negative no in-between interpretation. Thus above certain limit all well become positive and micro array hit upper limit. On the other hand below certain limit all well become negative and we hit lower limit.

SERS platforms integrated with multiple abilities such as filtration, concentration, capture, detection, and inactivation of bacteria were developed to allow for simultaneous application in real samples. Such applications use micro fluidic chips. Lower detection limit can go up to single cell. Use is reported for pathogens in blood samples as well as E. coli in food samples. Dielectrophoresis is use of non-uniform electric filed for separation of materials based on their dielectric constant and this technique can be successfully used for elective separation of cells or DNA in microfluidic platform [9].

Commercial systems are available based of Surface enhanced Raman scattering and they depends on analysis of signature spectra of bacteria i.e. they can be label free but many of them used antigen antibody like reaction for specific detection and use SERS in last stage for generation of signal. For example one can think of sandwich assay in which aptamer bound with gold nanoparticle bind with microorganism and another aptamer with Raman reporter is used to generate signal. If you want direct detection of pathogens without antibody like mechanism, sophisticated signal analysis is necessary. Use of signal database can help [10].

Specific Studies

Virus detection by traditional methods is laborious and time consuming. SERS with silver nanoparticles are used for detection of hepatitis B virus DNA at 50 aM level using antigen antibody reaction. Au/Ag core shell nanoparticles was used to detect influenza A virus as low as 5% infection rate causing concentration with help of antigen antibody reaction in conjugation with SERS [11].

SER spectroscopy and imaging was successfully used to detect pathogens in meat as per this review. SER imaging was used for spores and vegetative cell identification with help of silver nanoparticles [12].

Antibodies, aptamers and molecularly imprinted polymers can

be used with SERS for specificity and spores of *Bacillus anthracis* was detected using aptamers combined with SERS. Level of detection was 10E4 CFU/g and it took 40 minutes to do it. *S. typhimurium* detection is reported with aptamer-SERS at 4 CFU/ml in pork. E. coli, *Salmonella* and *Listeria* detection reported with aptamer SERS in beef and poultry with 10E3 to 10E6 CFU per gram detection limit. *Vibrio parahaemolyticus* was detected using aptramer SERS at 14 CFU/g limit in salmon [5].

- A magnetically-assisted surface-enhanced Raman scattering (SERS) biosensor has been developed for the recognition of *S. aureus* on the basis of aptamer recognition.
- Spores of *B. anthracis* can live after the pasteurization phase in food handling, thus is considered as a major risk in the field of food safety and defense. The aptamer-based SERS approach is able to quickly concentrate and distinguish the spores of this organism from orange juice. The limit of this detection technique in orange juice is 10E4 CFU spores per gram. The whole procedure can be performed within 40 min [13].

A SERS-aptasensor, using nanoparticles was established for the quantitative detection of *S. typhimurium* and *S. aureus* simultaneously with the detection limit of 35 CFU/ml for *S. aureus* and 15 CFU/ml for *S. typhimurium*. In this platform, gold nanoparticles modified with Raman molecules Mercaptobenzoic acid (MBA) and Ellman's Reagent (DNTB) and aptamers were applied as the signal probe. Thiolated aptamers of *S. typhimurium* and *S. aureus* are used as the capture probe with MBA and DNTP respectively, fixed on Fe_3O_4 magnetic gold nanoparticles. When after separation magnetic Fe@Au NPs came into contact with other GNPs with aptamers which bind with same microbes, lot of hot spot generated and Raman signal was out [14].

Gram positive bacteria *S. aureus* and *E. coli* was detected in milk at 10E3 cells per ml concentration using SERS. No antibody or any labels are used. Method used magnetic nanoparticles along with nonmagnetic concentrated Au@Ag nano particles to get enhanced Raman scattering. The magnetic nano particle was made up of Fe_3O_4@Au@Polyethylenimine and was positively charged. Negatively charged microbes are attracted to positively charged nanoparticles and enriched by magnetic separation. Later on Au@Ag nanao particle was added at detection stage as it gave synergic enhancement in signal. Method took only 10 minutes [15].

A label-free SERS detection of *E. coli* based on incubation with silver colloid was reported. The lowest concentration of *E. coli* at 10E5 cell/ml can be detected by SERS mapping. Three strains of *E. coli* DSM 1116/498/5695 could be successfully discriminated using such SERS method combining discriminant analysis [16].

A novel SERS enzyme-catalyzed immunoassay of Respiratory Syncytial Virus RSV by employing peroxidase substrate 3,3′–5,5′-tetramethylbenzidine (TMB) as Raman molecule was reported. Horseradish peroxidase (HRP) attached to the detection antibody in a novel sandwich immunoassay catalyses the oxidation of TMB by H_2O_2 to give a radical cation (TMB+), which could be easily adsorbed on the negatively charged surface of silver nanoparticles (AgNPs) through electrostatic interaction, inducing the aggregation of AgNPs and thus giving a strong SERS signal. A linear relationship was obtained between the Raman intensity and the amount of RSV in the range from 0.5 to 20 pg/ml, and the minimum detectable concentration of this SERS based enzyme immunoassay was 0.05 pg/ml, which was 20 times lower than that found in the colorimetric method [17].

DNA based detection method using a SERS nano platform for malaria diagnosis was reported. Core@reporter@shell type of nana particle used called nano rattles. Method involves sandwich hybridization of magnetic beads that are loaded with capture probes, target sequences, and ultra-bright SERS nanorattles that are loaded with reporter probes. Atto mol level detection limit reported [18].

Three different pure colonies of *Klebsiella pneumoniae* was grown and SERS technique was used for correct classification of samples using statistical analysis [19].

S. typhimurium detection was done by using two different aptamers. One aptamer was with Au@Ag nano particle and other was with Raman reporter molecule rhodamine. When both aptamer bound to bacterium they give SERS signal. The linear relationship between the SERS intensity and the *S. typhimurium* concentration was in the range from 15 to 1.5 10E6 CFU/ml [20].

Silver coated magnetic nano particles were used for detection of water borne pathogens. *Acinetobacter baylyi* and *E. coli* was detected with high sensitivity of 10E5 CFU/ml. No label or antibody was used and bacteria was absorbed on nano particles directly from water [21]. Note that water was not ordinary water but cell suspension mostly of pure culture.

A rapid and accurate surface-enhanced Raman spectroscopy (SERS) based on silver nanodots (AgNDs) array substrate method was used to detect *Alternaria alternata* (*A. alternata*) in both sterile water and pear juice (an example of complex food matrix). SERS mapping methods were then used to scan the *A. alternata* adsorbed on the surface of the substrate, and the intrinsic and distinct SERS signals of *A. alternata* were used as the basis for detection. It was found that using *A. alternata* in sterile water as a model sample, the method was able to detect the *A. alternata* with a LOD as low as 10E3 CFU/ml. Moreover, the newly developed method could also realize rapid detection of *A. alternata* in pear juice, and the lowest detectable *A. alternata* concentration was less than 10E4 CFU/ ml. In addition, the *A. alternata* concentration range from 10E4 CFU/ml to 10E6 CFU/ml could be detected by SERS mapping [22].

Nanoplate-bacteria-nanorod super crystals were reported to be highly effective sensors of bacteria using SERS signal due to very high enhancement of signal by hot spot mechanism. Successful detection and identification of bacteria in model samples consisting of two representative bacteria blends in soft-drink were demonstrated using newly developed super crystals combined with PCA analysis. Vancomycin was used as bacteria capturing glue [23].

A simple, low cost and rapid optical fingerprinting technology for clinical microorganism detection is demonstrated using AgNPs decorated filter-like SERS substrate combined with pattern recognition techniques. Statistical techniques are used to make classification of bacteria based on data. Use of filter was better than using raw nano particles. Three type of bacteria are tried [24].

Proof of concept detection of *E. coli* was performed using silicon nano pillars and silver nano particle based platform and SERS signal [25].

A highly selective and sensitive SERS-based aptasensor for the detection of *Vibrio parahaemolyticus* has been developed.

An effective SERS substrate was designed and fabricated by synthesis of SiO_2@Au NPs. To selectively detect *V. parahaemolyticus*, they employed aptamer 1 (apt 1) immobilized on the surface of SiO2@Au NPs. The Raman signal intensity is from a cyanine dye 3 (Cy3) modified aptamer 2 (apt 2), which binds to the target the same as SiO2@Au bound apt 1. In the presence of the target, SiO2@Au-apt 1-target-apt 2-Cy3 sandwich-like complexes form due to the high affinity and specificity of the aptamers. Under optimal conditions, the correlation between the SERS signal and the *V. parahaemolyticus* concentration is linear within a range of 10 to 10E6 CFU/ml with a limit of detection of 10 CFU/ml [26]. Research was done using pure cultures and cultures were not even mixed.

A simple, rapid, selective, and sensitive biosensor was constructed for detection of *Salmonella typhimurium*. The spiny GNPs were respectively functionalized with para-mercaptobenzoic acid Raman reporter and thiolated *S. typhimurium* aptamers as SERS nanoprobes. On the other side, the biotinylated aptamer was anchored on the microtiter plate which was coated with biotin binding Streptavidin protein layer. Range of quantification was 10 CFU/ml to 10E5 CFU/ml, with a limit of detection of 4 CFU/ml [27].

SERS coupled with principal component analysis can serve as a fast and reliable technique for detection and identification of dermatophyte fungi at both genus and species level. Ag coated SERS was directly procured form market. No antibody etc. used. Analysis was done by randomly picking pure colonies [28]. This was medical diagnosis oriented study.

Sandwich immunoassay method utilizing enzymatic activity of alkaline phosphatase (ALP) on 5- bromo-4-chloro-3-indolyl phosphate (BCIP) for *E. coli* detection was developed using SERS. For this purpose, spherical magnetic gold coated core-shell nanoparticles (MNPs-Au) and rod shape gold nanoparticles (Au-NRs) were synthesized. All nano particle were treated with 11 carbon long 11-mercaptoundecanoic acid. COOH group of this acid remain toward solution while thiol group is connected with gold. Magnetic particles were coated with antibody using biotin avidin mechanism. Biotin was attached with MNPs-Au and avidin was with antibody. Bovine serum albumin as used to coat surface of nano particles and thus avoid unwanted attachment. Rod shaped particles were coated with same things but in addition enzyme alkaline phosphate was also connected to it. *E. coli* bacteria was fist incubated with magnetic nano particles and separated by immune-magnetic separation (IMS). After separation rod shaped NPs are added to magnetic NPs. After that enzyme substrate BCIP was added and enzyme converted in to Raman reporter BCI which generated signal. A good (R^2 = 0.992) linear relation was observed between intensity of BCI at 600 cm^{-1} and logarithmic *E. coli* concentration in the range of 1.7 × 10E1 to 1.7 × 10E6 CFU/ml. LOD and LOQ values were also calculated and found to be 10 CFU/ml and 30 CFU/ml respectively [29].

SERS spectroscopy was used along with chemometric techniques to detect pathogens and identify them by their spectral profiles. Pathogens were grown in different cultivation conditions. Silver Nano Particles (SNPs) are either in situ synthesized on bacteria i.e. Bacteria@AgNPs or bacteria are incubated with preformed SNPs. No Raman reporters or antibody mechanism was used. Label-free detection and identification of the most common pathogens i.e. *E. coli*, *Aeromonas*, *M. morganii*, *E. lactis*, *L. casei* and *L. monocytogenes* was assessed. Method takes only 5 minutes for analysis [30]. Unusually this paper was lacking in information on key aspects i.e. material and methods and even conclusions!

The objective of this study was to characterize and evaluate a handheld SERS-based diagnostic system for the detection and identification of bacteria in pooled human sera. Species of *Acenitobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* were individually inoculated into pooled human serum samples. Samples were processed by lysis filtration to separate and isolate bacteria. Processed bacterial samples were incubated onto silver nanorod substrates at 60 °C for 3 h. Spectra of bacteria recovered from serum were compared to spectra of pure culture bacteria. PCA and PLSDA analysis were performed to determine bacterial "molecular fingerprint". Successful detection, identification, and classification of bacteria from human serum using a hand-held Raman spectrometer were demonstrated. Pure culture bacteria were readily identifiable and distinguishable by their SERS-based molecular fingerprints at the species level. Hydrophilic bacteria were readily detected and identified from serum samples without changes occurring to their spectra due to sample processing. Shifts in relative peak intensities of SERS spectra were observed primarily for hydrophobic bacteria after recovery from serum [31].

A microfluidic SERS based platform was developed for improved detection of pathogenic bacteria by using silver nanoparticles along with PCA and LDA statistical techniques. The method can distinguish eight key foodborne pathogens (*E. coli*, *S. typhimirium*, *S. enteritis*, *Pseudomonas aeruginosa*, *L. monocytogenes*, *L. innocua*, *MRSA 35* and *MRSA 86*) and, hence, holds good promise for use in the food industry as per authors. Pure bacterial suspension was used to collect data [32].

Fast and sensitive SERS method for the determination of *Mycobacterium smegmatis* is reported. It is based on the formation of AgNPs directly on the surface of bacteria via the silver mirror reaction. After this directly spectra is obtained. Method was also applied to detect *M. bovis BCG*, *M. tuberculosis*, *Staphylococcus aureus*, *S. epidermis*, *Bacillus cereus* and two laboratory strains of *Escherichia coli*, thus demonstrating the wider applicability of this approach. Pure bacterial cultures were used as samples [33].

SERS nanoprobe for the specific detection of *E. coli O157:H7* was reported. AuNPs were coated with a thick silica shell, and the Raman reporter (4,4′- dipyridyl) was embedded between gold nanoparticle and silica shell. This technique prevents any external effects on the AuNPs from the environment and avoids any interaction between the Raman reporter and possible impurities. The SERS nano particles remained stable for up to 50 hours. The limit of detection is as low as 10 CFU/ml. The technique was successfully employed to the detection of *E. coli O157:H7* in spiked river water, achieving recoveries between 95.5 and 114.8% [34].

A label-free method based on aptamer binding and SERS has been developed for the detection of *Salmonella typhimurium*. Surface of the SERS-active silver nanorod array substrates was modified with anti *S. typhimurium* DNA aptamer and mercaptohexanol, and reacted with *S. typhimurium* and *S. enteritidis*, *E. coli*, and *E. faecalis* as negative control bacteria. Changes in spectra are noticed between analysed bacteria and PCA analysis was confirmed this. High detection limit was observed due to unknown reasons 10E8 CFU/ml [35].

The detection of *Salmonella poona* from cantaloupe cubes

and *E. coli O157:H7* from lettuce has been explored by using a filtration method and SERS based on vancomycin functionalized silver nano-rod array substrates. Filtration was done to first at 40 μm to remove all particulate matter and to pass bacteria and second time filtration is done at 22 μm to retain the bacteria. Bacteria were recovered form second filter. It is found that with a two-step filtration process, the LOD of *Salmonella poona* from cantaloupe cubes can be as low as 100 CFU/ml in less than 4 h, whereas the chlorophyll in the lettuce causes severe SERS spectral interference. To improve the LOD of lettuce, a three-step filtration method with a third hydrophobic filter 22 μm is proposed. Chlorophyll passed from filter and only bacteria retained which are used for detection. The hydrophobic filter can effectively eliminate the interferences from chlorophyll and achieve a LOD of 1000 CFU/ml detection of *E. coli O157:H7* from lettuce samples within 5 hours [36].

A SERS response coming from *S. typhimurium* aptamers (ST aptamer), complementary DNA (cDNA), p-aminothiophenol (PATP) Raman reporter, and Au nanorods (GNRs) for sensitive detection of foodborne pathogens is reported. cDNA is complimentary DNA and in this case it is complimentary to aptamer to *S. typhimurium* (ST). GNRs are unstable in presence of salt and form clumps and thus hot spots and give signal if salt is present. The signal will be of anything which is entrapped with GNR clumps. ST aptamer provides protection to GNR when it is added to solution as it forms charged layer on GNRs by adsorption. Either cDNA or ST addition promote clumping and addition of both give synergistic effect. Both cDNA and ST was used in study and obtained strong signal was taken as measurement of ST count. Under optimal conditions, the SERS intensity was observed to increase linearly with ST concentration from 56 to 56 × 10E7 CFU/ml (R^2 = 0.971) with a LOD of 9 CFU/ml. Additionally, this aptasensor exhibits a high selectivity to other similar pathogens, and the ability of the method to detect ST was also confirmed in adulterated milk samples [37]. Note: Study was convoluted with not even clear conclusion on what is happening form authors. Schematic diagram they reported do not match with text.

Simultaneous detection and serotyping of *Salmonellae* by immunomagnetic separation and label-free SERS was reported. After immunomagnetic separation (IMS) and overnight culture, SERS spectra were collected from multiple replicates and experiments and analyzed by chemometrics. 65 to 100% correct identification and serotyping reported on unknown samples [38].

4-mercaptophenylboronic acid (4-MPBA) contains two functional groups: a thiol group that can strongly bind with Ag or Au and a boronic acid group that can binds with peptidoglycan in bacterial cell wall through the covalent bonds. In this study 4-MPBA coated silver dendrites were used as SERS substrate. No other antibody like molecule or reporter molecule used. For *Salmonella enterica* subsp *entericaas, Escherichia coli, Listeria monocytogenes* and *Lactococcus lactis* as low as 10E3 CFU/ml bacterial cells in 50 mM NH_4HCO_3 solution and 10E2 CFU/ml cells in both 1% casein and skimmed milk was detected as well as differentiated. Cells were added intentionally in media and then detected. Skim milk used was commercial skim milk. For SERS measurements, 10 μl of 4-MPBA functionalized Ag dendrites was mixed with 1 ml of the prepared bacterial cells suspension and incubated for 30 min under gentle shaking at room temperature to capture the bacterial cells. After incubation, the supernatant was removed with pipette and the Ag dendrites were washed twice with sterile double distilled water and dried and spectra was measured on dried product [39].

Escherichia coli O157:H7 was detected up to 1 cell/ml level. Three different SERS-tagged molecular probes targeting different epitopes of the *E. coli* used simultaneously and this was the reason of lower detection limit. It was also possible to identify sub species. Statistical tools were used to interpret data. Detection was done on microfluidic system and dielectrophoretic enrichment was employed. Gold nano rods (GNRs) are used with 4 ATP and ATT Raman reporters to make GNR-ATP and GNR-ATT assemblies. Four different antibodies were used two with GNR-ATP and two with GNR-ATT. In addition to this 3-Mercaptopropionic gold nano cages with another antibodies were used [40].

Food pathogen *Vibrio parahaemolyticus* was detected using SERS. Nanostructures consisting of Fe_3O_4@Au particles wrapped with graphene oxide (GO) were used both as SERS substrates and separation tools. GO has the ability to quench molecule fluorescence to improve the Raman signal to noise ratio. A first aptamer (apt 1) was immobilized on the Fe_3O_4@Au/GO nanostructures to act as a capture probe via the affinity binding of aptamer and *V. parahaemolyticus*. A second aptamer (apt-2) was modified with the Raman reporter molecule TAMRA to act as a SERS sensing probes that binds to the target the same way as the Fe_3O_4@Au/GO-apt 1. The sandwich formed between Fe_3O_4@Au/GO-apt 1 @ *V. parahaemolyticus* @ apt 2-TAMRA can be separated with the aid of a magnet. The concentration of *V. parahaemolyticus* can be quantified by measurement of the SERS intensity of TAMRA. Detection limit was 14 CFU/ml. The method was successfully tested on spiked salmon samples [41].

More recentsly food pathogen Staphylococcus aureus detection using SERS and aptamer along with enzyme was reported and found to giving similar results to plate counting [42]. Use of antibodies along with organic and inorganic hybrid Au/Fe3+ magnetic nano-clusters was reported to successfully detect *E. coli* O157:H7 at 2 CFU/ml level [43].

Advantages of SERS

Pathogen detection may take a week by traditional culture and plating methods. It is difficult to detect single cell per ml using even ELISA and PCR but SERS can do it. The method can be used in field condition as once substrate is ready sample preparation may not be required and portable instruments are available.

Limitations of SERS

In many detection methods selective attachment of microorganisms is done which requires use of antibody like mechanisms and this is limitation of SERS method. Note that selective attachment to surface is not essential and direct identification is possible due to finger print properties of Raman spectra. For complex mixtures too much noise is problem i.e. thousands of identical microbes and thus no identifiable signal for target microbe. At extreme the signal may average out and you see just random noise. SER substrates can be fine-tuned to enhance particular signal which helps in removing noise. SERS is more sensitive suitable to small size molecules or things and IR spectroscopy is more suitable for larger molecules due to strong distance dependence on enhancement in SERS. Detection limits may be lower if antibody like specific binding mechanism is used

instead of depending of non-specific binding or no binding particularly in presence of noise.

For nano particle based assays reproducibility of signal is problem. As every time particle agglomeration, size of particles, distribution of particles on microbe, experimental environment etc. may differ and you may get different signal making it useless. Enhancement of Raman signal depend on geometry of surface and thus we have to run standards every time for quantification.

In addition, the currently available SERS biosensors require bulky instruments, such as optical microscopes, lasers, monochromators, and detectors [44]. Hand held instruments suffer from low sensitivity in relation to bench top machines.

Lack of a comprehensive mechanism or database of SERS band assignments will make the process of annotating spectra less valuable than it would be otherwise. ❖ Quantification is more difficult than detection. ❖ Though nanostructured metal surface based method can provide a relatively stable and reproducible SERS signal, the complicated synthesis and high cost limit its application [9].

Conclusion

SERS is sensitive technique which can detect at the level of even single cell per ml which is difficult with even PCR or ELISA. Once Raman substrate is ready sample preparation is minimal compared to other techniques and detection is rapid. Quantification is difficult in relation to detection and requires standards and some methods suffer from reproducibility issues. Decreasing cost of electronics and availability of handled devices opened new frontiers for this technique and it can be used in field settings for rapid analysis of pathogens.

Bibliography

1. Fan M, Andrade GFS, Brolo AG. A review on the fabrication of substrates for surface enhanced Raman spectroscopy and their applications in analytical chemistry. Analytica Chimica Acta 2011;693(1):7-25. doi:10.1016/j.aca.2011.03.002
2. Li W, Zhao X, Yi Z, Glushenkov AM, Kong L. Plasmonic substrates for surface enhanced Raman scattering. Analytica Chimica Acta 2017;984:19-41. doi:10.1016/j.aca.2017.06.002
3. Sharma B, Cardinal MF, Kleinman SL *et al*. High-performance SERS substrates: Advances and challenges. MRS Bulletin 2013;38(8):615-624. doi:10.1557/mrs.2013.161
4. Yuan Y, Panwar N, Yap SHK *et al*. SERS-based ultrasensitive sensing platform: An insight into design and practical applications. Coordination Chemistry Reviews 2017;337:1-33. doi:10.1016/j.ccr.2017.02.006
5. Yaseen T, Pu H, Sun DW. Functionalization techniques for improving SERS substrates and their applications in food safety evaluation: A review of recent research trends. Trends in Food Science & Technology 2018;72:162-174. doi:10.1016/j.tifs.2017.12.012
6. Zeng Z, Liu Y, Wei J. Recent advances in surface-enhanced raman spectroscopy (SERS): Finite-difference time-domain (FDTD) method for SERS and sensing applications. TRAC Trends in Analytical Chemistry 2016;75:162-173. doi:10.1016/j.trac.2015.06.009
7. Jiang Y, Sun DW, Pu H, Wei Q. Surface enhanced Raman spectroscopy (SERS): A novel reliable technique for rapid detection of common harmful chemical residues. Trends in Food Science & Technology 2018;75:10-22. doi:10.1016/j.tifs.2018.02.020
8. Sharma B, Frontiera RR, Henry AI, Ringe E, Van Duyne RP. SERS: Materials, applications, and the future. Materials Today 2012;15(1):16-25. doi:10.1016/S1369-7021(12)70017-2
9. Liu Y, Zhou H, Hu Z, Yu G, Yang D, Zhao J. Label and label-free based surface-enhanced Raman scattering for pathogen bacteria detection: A review. Biosensors and Bioelectronics 2017;94:131-140. doi:10.1016/j.bios.2017.02.032
10. Yoo SM, Lee SY. Optical Biosensors for the Detection of Pathogenic Microorganisms. Trends in Biotechnology 2016;34(1):7-25. doi:10.1016/j.tibtech.2015.09.012
11. Mokhtarzadeh A, Eivazzadeh-Keihan R, Pashazadeh P *et al*. Nanomaterial-based biosensors for detection of pathogenic virus. TRAC Trends in Analytical Chemistry 2017;97:445-457. doi:10.1016/j.trac.2017.10.005
12. Yaseen T, Sun DW, Cheng JH. Raman imaging for food quality and safety evaluation: Fundamentals and applications. Trends in Food Science & Technology 2017;62:177-189. doi:10.1016/j.tifs.2017.01.012
13. Alizadeh N, Memar MY, Moaddab SR, Kafil HS. Aptamer-assisted novel technologies for detecting bacterial pathogens. Biomedicine & Pharmacotherapy 2017;93:737-745. doi:10.1016/j.biopha.2017.07.011
14. Ansari N, Yazdian-Robati R, Shahdordizadeh M, Wang Z, Ghazvini K. Aptasensors for quantitative detection of Salmonella Typhimurium. Analytical Biochemistry 2017;533:18-25. doi:10.1016/j.ab.2017.06.008
15. Wang C, Wang J, Li M *et al*. A rapid SERS method for label-free bacteria detection using polyethylenimine-modified Au-coated magnetic microspheres and Au@Ag nanoparticles. Analyst 2016;141(22):6226-6238. doi:10.1039/C6AN01105E
16. Yang D, Zhou H, Haisch C, Niessner R, Ying Y. Reproducible E. coli detection based on label-free SERS and mapping. Talanta 2016;146:457-463. doi:10.1016/j.talanta.2015.09.006
17. Zhan L, Zhen SJ, Wan XY, Gao PF, Huang CZ. A sensitive surface-enhanced Raman scattering enzyme-catalyzed immunoassay of respiratory syncytial virus. Talanta 2016;148:308-312. doi:10.1016/j.talanta.2015.10.081
18. Ngo HT, Gandra N, Fales AM, Taylor SM, Vo-Dinh T. Sensitive DNA detection and SNP discrimination using ultrabright SERS nanorattles and magnetic beads for malaria diagnostics. Biosensors and Bioelectronics 2016;81:8-14. doi:10.1016/j.bios.2016.01.073
19. Cheong Y, Kim YJ, Kang H, Choi S, Lee HJ. Rapid label-free identification of Klebsiella pneumoniae antibiotic resistant strains by the drop-coating deposition surface-enhanced Raman scattering method. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 2017;183:53-59. doi:10.1016/j.saa.2017.04.044
20. Duan N, Chang B, Zhang H, Wang Z, Wu S. Salmonella typhimurium detection using a surface-enhanced Raman scattering-based aptasensor. International Journal of Food Microbiology 2016;218:38-43. doi:10.1016/j.ijfoodmicro.2015.11.006
21. Li H, Li C, Martin FL, Zhang D. Diagnose Pathogens in Drinking Water via Magnetic Surface-Enhanced Raman Scattering (SERS) Assay. Materials Today: Proceedings 2017;4(1):25-31. doi:10.1016/j.matpr.2017.01.189

22. Pan T, Sun DW, Pu H, Wei Q, Xiao W, Wang QJ. Detection of A. alternata from pear juice using surface-enhanced Raman spectroscopy based silver nanodots array. Journal of Food Engineering 2017;215:147-155. doi:10.1016/j.jfoodeng.2017.07.019
23. Wang W, Hynninen V, Qiu L *et al*. Synergistic enhancement via plasmonic nanoplate-bacteria-nanorod supercrystals for highly efficient SERS sensing of food-borne bacteria. Sensors and Actuators B: Chemical 2017;239:515-525. doi:10.1016/j.snb.2016.08.040
24. Lin CC, Lin CY, Kao CJ, Hung CH. High efficiency SERS detection of clinical microorganism by AgNPs-decorated filter membrane and pattern recognition techniques. Sensors and Actuators B: Chemical 2017;241:513-521. doi:10.1016/j.snb.2016.09.183
25. Karadan P, Aggarwal S, Anappara AA, Narayana C, Barshilia HC. Tailored periodic Si nanopillar based architectures as highly sensitive universal SERS biosensing platform. Sensors and Actuators B: Chemical 2018;254:264-271. doi:10.1016/j.snb.2017.07.088
26. Duan N, Yan Y, Wu S, Wang Z. Vibrio parahaemolyticus detection aptasensor using surface-enhanced Raman scattering. Food Control 2016;63:122-127. doi:10.1016/j.foodcont.2015.11.031
27. Ma X, Xu X, Xia Y, Wang Z. SERS aptasensor for Salmonella typhimurium detection based on spiny gold nanoparticles. Food Control 2018;84:232-237. doi:10.1016/j.foodcont.2017.07.016
28. Witkowska E, Jagielski T, Kamińska A. Genus- and species-level identification of dermatophyte fungi by surface-enhanced Raman spectroscopy. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 2018;192:285-290. doi:10.1016/j.saa.2017.11.008
29. Bozkurt AG, Buyukgoz GG, Soforoglu M, Tamer U, Suludere Z, Boyaci IH. Alkaline phosphatase labeled SERS active sandwich immunoassay for detection of Escherichia coli. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 2018;194:8-13. doi:10.1016/j.saa.2017.12.057
30. Dina NE, Colniță A, Leopold N, Haisch C. Rapid Single-cell Detection and Identification of Bacteria by Using Surface-enhanced Raman Spectroscopy. Procedia Technology 2017;27:203-207. doi:10.1016/j.protcy.2017.04.086
31. Kotanen CN, Martinez L, Alvarez R, Simecek JW. Surface enhanced Raman scattering spectroscopy for detection and identification of microbial pathogens isolated from human serum. Sensing and Bio-Sensing Research 2016;8:20-26. doi:10.1016/j.sbsr.2016.03.002
32. Mungroo NA, Oliveira G, Neethirajan S. SERS based point-of-care detection of food-borne pathogens. *Microchim Acta* 2016;183(2):697-707. doi:10.1007/s00604-015-1698-y
33. Alula MT, Krishnan S, Hendricks NR, Karamchand L, Blackburn JM. Identification and quantitation of pathogenic bacteria via in-situ formation of silver nanoparticles on cell walls, and their detection via SERS. Microchim Acta 2017;184(1):219-227. doi:10.1007/s00604-016-2013-2
34. Zhu T, Hu Y, Yang K, Dong N, Yu M, Jiang N. A novel SERS nanoprobe based on the use of core-shell nanoparticles with embedded reporter molecule to detect E. coli O157:H7 with high sensitivity. Mikrochim Acta 2017;185(1):30. doi:10.1007/s00604-017-2573-9
35. Chen J, Park B, Huang Y, Zhao Y, Kwon Y. Label-free SERS detection of Salmonella Typhimurium on DNA aptamer modified AgNR substrates. Food Measure 2017;11(4):1773-1779. doi:10.1007/s11694-017-9558-6
36. Wu X, Han C, Chen J, Huang YW, Zhao Y. Rapid Detection of Pathogenic Bacteria from Fresh Produce by Filtration and Surface-Enhanced Raman Spectroscopy. JOM 2016;68(4):1156-1162. doi:10.1007/s11837-015-1724-x
37. Li H, Chen Q, Ouyang Q, Zhao J. Fabricating a Novel Raman Spectroscopy-Based Aptasensor for Rapidly Sensing Salmonella typhimurium. Food Anal Methods 2017;10(9):3032-3041. doi:10.1007/s12161-017-0864-8
38. Chen J, Park B, Eady M. Simultaneous Detection and Serotyping of Salmonellae by Immunomagnetic Separation and Label-Free Surface-Enhanced Raman Spectroscopy. Food Anal Methods 2017;10(9):3181-3193. doi:10.1007/s12161-017-0870-x
39. Wang P, Pang S, Pearson B *et al*. Rapid concentration detection and differentiation of bacteria in skimmed milk using surface enhanced Raman scattering mapping on 4-mercaptophenylboronic acid functionalized silver dendrites. Anal Bioanal Chem 2017;409(8):2229-2238. doi:10.1007/s00216-016-0167-8
40. Wang C, Madiyar F, Yu C, Li J. Detection of extremely low concentration waterborne pathogen using a multiplexing self-referencing SERS microfluidic biosensor. J Biol Eng 2017;11(1):9. doi:10.1186/s13036-017-0051-x
41. Duan N, Shen M, Wu S, Zhao C, Ma X, Wang Z. Graphene oxide wrapped Fe3O4@Au nanostructures as substrates for aptamer-based detection of Vibrio parahaemolyticus by surface-enhanced Raman spectroscopy. Microchimica Acta. 2017;184(8):2653-2660. doi:10.1007/s00604-017-2298-9
42. Liu S, Li H, Hassan MM, Ali S, Chen Q. SERS based artificial peroxidase enzyme regulated multiple signal amplified system for quantitative detection of foodborne pathogens. Food Control 2021;123:107733. doi:10.1016/j.foodcont.2020.107733
43. Li Y, Gao F, Lu C, Fauconnier ML, Zheng J. Bio-Specific Au/Fe3+ Porous Spongy Nanoclusters for Sensitive SERS Detection of Escherichia coli O157:H7. Biosensors 2021;11(10):354. doi:10.3390/bios11100354
44. Rubab M, Shahbaz HM, Olaimat AN, Oh DH. Biosensors for rapid and sensitive detection of Staphylococcus aureus in food. Biosensors and Bioelectronics 2018;105:49-57. doi:10.1016/j.bios.2018.01.023