SERS for the Detection of Foodborne Pathogenic Bacteria

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Detection of foodborne pathogens at an early stage is very important to control food quality and improve medical response. Rapid detection of foodborne pathogens with high sensitivity and specificity is becoming an urgent requirement in health safety, medical diagnostics, environmental safety, and controlling food quality. Surface-enhanced Raman spectroscopy (SERS) is a real-time detection method that depends on the inelastic scattering of excitation light and molecular resonance, and has been applied to different applications for detecting and classifying various pathogens.

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1. Introduction

Foodborne pathogens cause diseases that affect both human health and the economy. Food and water are an essential part of life, and their contamination by bacteria poses a serious threat to human health and lifestyles [1][2]. Food-industry operators require rapid testing devices to monitor the quality of food for the presence of pathogenic bacteria [3]. Every year, millions of people worldwide get infected by contaminated food and water by microorganisms that cause various diseases. It is estimated that around 600 million foodborne diseases occur annually, with a mortality rate of 420,000 [4]. The Centers for Disease Control and Prevention estimated that approximately 2.5 billion people lack access to healthy and safe water in developing nations. Every year, more than 2.2 million mortality rates are reported due to waterborne diseases [5]. In China, a summary of studies estimated that the prevalence of pathogens in the food was 8.5% [6]. Controlling food safety is a persistent and challenging task in China due to the diversity of foods and food-production industries. Microbes such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enterica*, and *Listeria monocytogenes* are usually highly infectious, and the presence of few colony-forming units (CFUs) can cause disease [7]. Therefore, it is crucial to identify pathogens at an initial stage with highly sensitive techniques to avoid diseases and outbreaks [8][9].

Conventional bacteria detection methods include cultivation, Gram staining, and biochemical analysis. These methods are reliable and have made great contributions to pathogen detection, but they are time-consuming and often take 2 to 3 days or more, which is not convenient for the rapid identification of microbes. The current techniques that are being used as clinical methods include polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), and matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry [10][11][12][13][14]. The latest techniques have revolutionized the field of diagnosis due to high sensitivity and specificity. However, some of the drawbacks associated with current clinical diagnostic techniques include high

cost, controlled sampling conditions, being laborious and time-consuming, and the requirement for a skilled operator. Therefore, further research progress is required to develop a user-friendly, portable, and economic diagnostic development system. The development of simple techniques and systems for the rapid, economical, and on-site analytical approaches are essential in public health safety, medical diagnostics, and food safety [15][16][17][18][19].

2. Surface-Enhanced Raman Spectroscopy (SERS)

Surface-enhanced Raman spectroscopy is a real-time detection method that depends on the inelastic scattering of excitation light and molecular resonance. SERS inherits the significant chemical fingerprint information on Raman spectroscopy and enhances sensitivity using plasmon-enhanced excitation and scattering. In SERS, the inelastic scattering from the molecules is greatly enhanced by a factor of up to 10^8 when the molecules are adsorbed onto corrugated metal surfaces. The method can rapidly and efficiently detect a range of chemical structures and material compositions with high accuracy and reproducibility. These advantages make SERS a very promising tool for developing microbial detection techniques. SERS has been applied to different applications for detecting and classifying various pathogens [20][21][22][23][24].

Wang et al. developed a surface-enhanced Raman scattering (SERS)-based LFA strip for the detection of such pathogens as *Yersinia pestis*, *Francisella tularensis*, and *Bacillus anthracis*. Target-specific SERS nanotags (Raman reporter-labeled gold nanoparticles) were utilized instead of gold nanoparticles. The method detected the pathogens in a short duration of 15 min using a minimum sample volume of 40 µL. The obtained detection limits for *Y. pestis*, *F. tularensis*, and *B. anthracis* were 43.4 CFU/mL, 45.8 CFU/mL, and 357 CFU/mL, respectively [25]. A high-quality silver nanorod (AgNR)-based SERS substrate was prepared to acquire the chemical fingerprint information of 22 strains of common pathogens. The method was able to identify and discriminate 20 strains of pathogens (diluted to 10^7 CFU/mL) with high sensitivity within 30 min [26]. Another SERS-based biosensor was fabricated using gold nanorods (GNRs) complexed with oligonucleotide aptamers. The SERS tags were combined with antibody-modified magnetic nanoparticles for the simultaneous detection of *Escherichia coli* and *Salmonella typhimurium*. The developed SERS biosensor showed a good linear response of 10^1 to 10^6 CFU/mL, high detection sensitivity (<8 CFU/mL) and a recovery rate of 95.26–107.88%. That study on combining aptamers and Raman reporters in SERS tags makes it possible to simultaneously detect different pathogens using a single biosensor [27].

Artificial intelligence has been applied widely in different diagnostics applications. Machine learning and neural networks are emerging techniques for data analysis and classification [28]. Spectroscopy data acquired from the SERS biosensors and techniques have been applied in machine learning and neural network algorithms. Ding et al. developed a method by combining SERS with a multiscale convolutional neural network (CNN). The label-free Raman substrate was prepared using gold nanoparticles. Different 1854 SERS spectra of three *Salmonella* serovars were measured and a multiscale CNN model was applied to extract SERS spectral features. The prepared gold nanoparticles and the developed CNN model showed detection accuracy higher than 97%. The given outcomes showed that the combination of SERS spectroscopy with multiscale CNN is feasible for *Salmonella*

serotyping (*S. enteritidis*, *S. typhimurium*, and *S.* Paratyphi) with bacterial concentration of 10^8 CFU/mL [29]. A stacked autoencoder-based deep neural networks algorithm was applied using SERS for the detection of methicillin-resistant *Staphylococcus aureus* and methicillin-sensitive *S. aureus.* The developed algorithm can evaluate features from the acquired signals and classify the data with an accuracy of 97.99%. The developed deep learning model classifies the pathogens with an area under the curve of 0.99 [30]. Ciloglu et al. combined SERS with machine learning techniques to classify *Staphylococcus aureus* and *Legionella pneumophila*. The technique gives higher classification accuracy of 97.8% by applying the *k*-nearest neighbors classifier [31].

Raman spectroscopy has been utilized extensively for microbiological diagnostics. A point-of-care testing technique has been developed using adhesive tape as a single platform for fast sampling, photocontrolled release, and SERS detection of pathogens from infected wounds. Pathogenic infections of *P. aeruginosa* and *S. aureus* were detected using gold nanostars on the adhesive tape as SERS substrate. The detection limit of the technique is 1.8 nM [32]. Duan et al. developed a SERS aptasensor for simultaneous detection of various pathogens using gold-decorated PDMS substrate. The fabricated film bound with the SERS probe to detect *Vibrio parahaemolyticus* and *Salmonella typhimurium* with a selectively detection limit of 18 CFU/mL and 27 CFU/mL, respectively [33]. The advancement of materials in SERS technology has increased accuracy and sensitivity for the detection of pathogens. The binding of SERS probes with fabricated chips and PDMS materials has enabled continuously miniaturization of detection prototypes [34][35].

Nakar et al. obtained spectra from pathogens *E. coli*, *Klebsiella pneumoniae*, and *Klebsiella oxytoca* isolates using UV-resonance Raman spectroscopy and single-cell Raman microspectroscopy. The obtained spectra were analyzed by machine learning algorithms for the classification of bacteria at the genus and species levels. The technique provides higher classification with 92% accuracy [36]. The method was further applied for the detection of clinical strains of *E. coli* [37]. Shen et al. created a fiber-probe-based method of Raman spectroscopy for the identification of six pathogens (*S. epidermidis*, *S. aureus*, *E. faecalis*, *E. faecium*, *P. aeruginosa*, and the yeast *C. albicans*). The collected signals from the fiber probe were analyzed using principal component analysis and linear discrimination models. The classification model acquired results with an accuracy of 93.8% [38]. The given studies were further extended and applied on agar plates to classify pathogenic infections [39].

SERS scattering has been incorporated with a microfluidic chip for the identification and discrimination of pathogens using tagged gold nanostars. The testing sample flowed continuously through the microfluidic channel, and the SERS signal was acquired corresponding to the SERS-tagged nanostars coated with antibody-binding protein. The system is capable of discriminating between *L. monocytogenes* and *Listeria innocua* with a concentration of 10^5 CFU/mL. Analyzing the data for the detection of pathogens requires less than 2 min. However, overall sample preparation and system operation time requires 30 min. A schematic illustration of the on-chip detection of *L. monocytogenes* using SERS can be found in [40]. Bai et al. developed a sandwich immunoassay platform using functionalized SERS probes and magnetic beads for the simultaneous detection of *E. coli* and *S. aureus*. The technique uses two SERS probes for acquiring the signal following the immunomagnetic separation of the sample. The method identifies the pathogen with a detection limit of 10 and 25 CFU/mL for the simultaneous detection of *E. coli* and *S. aureus*, respectively [41]. Overall, SERS has emerged as a powerful analytical tool for

rapidly detecting pathogens. Recent progress in the field of micro- to nanofabrication methodologies has enabled SERS applicable to various applications, such as rapid detection, point-of-care detection, and in situ detection. Currently, commercially available pathogen-detection techniques using SERS do not yet exist, but the improvement in SERS techniques has made it possible to develop handheld and portable prototypes to detect pathogens rapidly.

References

1. Tan, X.-B.; Zhao, X.-C.; Zhang, Y.-L.; Zhou, Y.-Y.; Yang, L.-B.; Zhang, W.-W. Enhanced lipid and biomass production using alcohol wastewater as carbon source for Chlorella pyrenoidosa cultivation in anaerobically digested starch wastewater in outdoors. Bioresour. Technol. 2018, 247, 784–793.
2. Kumar, H.; Kuča, K.; Bhatia, S.K.; Saini, K.; Kaushal, A.; Verma, R.; Bhalla, T.C.; Kumar, D. Applications of Nanotechnology in Sensor-Based Detection of Foodborne Pathogens. Sensors 2020, 20, 1966.
3. Foddai, A.C.G.; Grant, I.R. Methods for detection of viable foodborne pathogens: Current state-of-art and future prospects. Appl. Microbiol. Biotechnol. 2020, 104, 4281–4288.
4. Xing, G.; Zhang, W.; Li, N.; Pu, Q.; Lin, J.-M. Recent progress on microfluidic biosensors for rapid detection of pathogenic bacteria. Chin. Chem. Lett. 2022, 33, 1743–1751.
5. Bhardwaj, N.; Bhardwaj, S.K.; Bhatt, D.; Lim, D.K.; Kim, K.-H.; Deep, A. Optical detection of waterborne pathogens using nanomaterials. TrAC Trends Anal. Chem. 2019, 113, 280–300.
6. Paudyal, N.; Pan, H.; Liao, X.; Zhang, X.; Li, X.; Fang, W.; Yue, M. A Meta-Analysis of Major Foodborne Pathogens in Chinese Food Commodities Between 2006 and 2016. Foodborne Pathog. Dis. 2018, 15, 187–197.
7. Ma, Y.; Ding, S.; Fei, Y.; Liu, G.; Jang, H.; Fang, J. Antimicrobial activity of anthocyanins and catechins against foodborne pathogens Escherichia coli and Salmonella. Food Control 2019, 106, 106712.
8. Manole, E.; Dumitrescu, L.; Niculițe, C.; Popescu, B.O.; Ceafalan, L.C. Potential roles of functional bacterial amyloid proteins, bacterial biosurfactants and other putative gut microbiota products in the etiopathogeny of Parkinson’s Disease. BIOCELL 2021, 45, 1–16.
9. Al-Tayyar, N.A.; Youssef, A.M.; Al-Hindi, R.R. Edible coatings and antimicrobial nanoemulsions for enhancing shelf life and reducing foodborne pathogens of fruits and vegetables: A review. Sustain. Mater. Technol. 2020, 26, e00215.
10. Hameed, S.; Xie, L.; Ying, Y. Conventional and emerging detection techniques for pathogenic bacteria in food science: A review. Trends Food Sci. Technol. 2018, 81, 61–73.

11. Li, H.; Bai, R.; Zhao, Z.; Tao, L.; Ma, M.; Ji, Z.; Jian, M.; Ding, Z.; Dai, X.; Bao, F.; et al. Application of droplet digital PCR to detect the pathogens of infectious diseases. Biosci. Rep. 2018, 38, BSR20181170.
12. Xiong, Y.; Leng, Y.; Li, X.; Huang, X.; Xiong, Y. Emerging strategies to enhance the sensitivity of competitive ELISA for detection of chemical contaminants in food samples. TrAC Trends Anal. Chem. 2020, 126, 115861.
13. Ye, J.; Guo, J.; Li, T.; Tian, J.; Yu, M.; Wang, X.; Majeed, U.; Song, W.; Xiao, J.; Luo, Y.; et al. Phage-based technologies for highly sensitive luminescent detection of foodborne pathogens and microbial toxins: A review. Compr. Rev. Food Sci. Food Saf. 2022, 21, 1843–1867.
14. Chen, X.-F.; Hou, X.; Xiao, M.; Zhang, L.; Cheng, J.-W.; Zhou, M.-L.; Huang, J.-J.; Zhang, J.-J.; Xu, Y.-C.; Hsueh, P.-R. Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) Analysis for the Identification of Pathogenic Microorganisms: A Review. Microorganisms 2021, 9, 1536.
15. Tang, Y.; Ali, Z.; Dai, J.; Liu, X.; Wu, Y.; Chen, Z.; He, N.; Li, S.; Wang, L. Single-Nucleotide Polymorphism Genotyping of exoS in Pseudomonas aeruginosa Using Dual-Color Fluorescence Hybridization and Magnetic Separation. J. Biomed. Nanotechnol. 2018, 14, 206–214.
16. Ling, Y.; Zhu, Y.; Fan, H.; Zha, H.; Yang, M.; Wu, L.; Chen, H.; Li, W.; Wu, Y.; Chen, H. Rapid Method for Detection of Staphylococcus aureus in Feces. J. Biomed. Nanotechnol. 2019, 15, 1290–1298.
17. Wang, K.; Wang, Z.; Zeng, H.; Luo, X.; Yang, T. Advances in Portable Visual Detection of Pathogenic Bacteria. ACS Appl. Bio Mater. 2020, 3, 7291–7305.
18. He, L.; Yang, H.; Xiao, P.; Singh, R.; He, N.; Liu, B.; Li, Z. Highly Selective, Sensitive and Rapid Detection of Escherichia coli O157:H7 Using Duplex PCR and Magnetic Nanoparticle-Based Chemiluminescence Assay. J. Biomed. Nanotechnol. 2017, 13, 1243–1252.
19. Wang, C.; Liu, M.; Wang, Z.; Li, S.; Deng, Y.; He, N. Point-of-care diagnostics for infectious diseases: From methods to devices. Nano Today 2021, 37, 101092.
20. Wang, P.; Sun, Y.; Li, X.; Wang, L.; Xu, Y.; He, L.; Li, G. Recent advances in dual recognition based surface enhanced Raman scattering for pathogenic bacteria detection: A review. Anal. Chim. Acta 2021, 1157, 338279.
21. Wang, K.; Li, S.; Petersen, M.; Wang, S.; Lu, X. Detection and Characterization of Antibiotic-Resistant Bacteria Using Surface-Enhanced Raman Spectroscopy. Nanomaterials 2018, 8, 762.
22. Zhou, X.; Hu, Z.; Yang, D.; Xie, S.; Jiang, Z.; Niessner, R.; Haisch, C.; Zhou, H.; Sun, P. Bacteria Detection: From Powerful SERS to Its Advanced Compatible Techniques. Adv. Sci. 2020, 7, 2001739.

23. Wang, C.; Meloni, M.M.; Wu, X.; Zhuo, M.; He, T.; Wang, J.; Wang, C.; Dong, P. Magnetic plasmonic particles for SERS-based bacteria sensing: A review. AIP Adv. 2019, 9, 010701.
24. Li, D.; Liu, L.; Huang, Q.; Tong, T.; Zhou, Y.; Li, Z.; Bai, Q.; Liang, H.; Chen, L. Recent advances on aptamer-based biosensors for detection of pathogenic bacteria. World J. Microbiol. Biotechnol. 2021, 37, 45.
25. Wang, R.; Kim, K.; Choi, N.; Wang, X.; Lee, J.; Jeon, J.H.; Rhie, G.-e.; Choo, J. Highly sensitive detection of high-risk bacterial pathogens using SERS-based lateral flow assay strips. Sensors Actuators B Chem. 2018, 270, 72–79.
26. Liu, S.; Hu, Q.; Li, C.; Zhang, F.; Gu, H.; Wang, X.; Li, S.; Xue, L.; Madl, T.; Zhang, Y.; et al. Wide-Range, Rapid, and Specific Identification of Pathogenic Bacteria by Surface-Enhanced Raman Spectroscopy. ACS Sensors 2021, 6, 2911–2919.
27. Li, Y.; Lu, C.; Zhou, S.; Fauconnier, M.-L.; Gao, F.; Fan, B.; Lin, J.; Wang, F.; Zheng, J. Sensitive and simultaneous detection of different pathogens by surface-enhanced Raman scattering based on aptamer and Raman reporter co-mediated gold tags. Sensors Actuators B Chem. 2020, 317, 128182.
28. Chen, Z.; Xiao, C.; Qiu, H.; Tan, X.; Jin, L.; He, Y.; Guo, Y.; He, N. Recent Advances of Artificial Intelligence in Cardiovascular Disease. J. Biomed. Nanotechnol. 2020, 16, 1065–1081.
29. Ding, J.; Lin, Q.; Zhang, J.; Young, G.M.; Jiang, C.; Zhong, Y.; Zhang, J. Rapid identification of pathogens by using surface-enhanced Raman spectroscopy and multi-scale convolutional neural network. Anal. Bioanal. Chem. 2021, 413, 3801–3811.
30. Ciloglu, F.U.; Caliskan, A.; Saridag, A.M.; Kilic, I.H.; Tokmakci, M.; Kahraman, M.; Aydin, O. Drug-resistant Staphylococcus aureus bacteria detection by combining surface-enhanced Raman spectroscopy (SERS) and deep learning techniques. Sci. Rep. 2021, 11, 18444.
31. Ciloglu, F.U.; Saridag, A.M.; Kilic, I.H.; Tokmakci, M.; Kahraman, M.; Aydin, O. Identification of methicillin-resistant Staphylococcus aureus bacteria using surface-enhanced Raman spectroscopy and machine learning techniques. Analyst 2020, 145, 7559–7570.
32. Guo, J.; Zhong, Z.; Li, Y.; Liu, Y.; Wang, R.; Ju, H. “Three-in-One” SERS Adhesive Tape for Rapid Sampling, Release, and Detection of Wound Infectious Pathogens. ACS Appl. Mater. Interfaces 2019, 11, 36399–36408.
33. Duan, N.; Shen, M.; Qi, S.; Wang, W.; Wu, S.; Wang, Z. A SERS aptasensor for simultaneous multiple pathogens detection using gold decorated PDMS substrate. Spectrochim. Acta A Mol. Biomol. Spectrosc. 2020, 230, 118103.
34. Zhao, X.; Li, M.; Xu, Z. Detection of Foodborne Pathogens by Surface Enhanced Raman Spectroscopy. Front. Microbiol. 2018, 9, 1236.

35. Lin, Z.; He, L. Recent advance in SERS techniques for food safety and quality analysis: A brief review. Curr. Opin. Food Sci. 2019, 28, 82–87.
36. Nakar, A.; Pistiki, A.; Ryabchykov, O.; Bocklitz, T.; Rösch, P.; Popp, J. Label-free differentiation of clinical E. coli and Klebsiella isolates with Raman spectroscopy. J. Biophotonics 2022, 15, e202200005.
37. Nakar, A.; Pistiki, A.; Ryabchykov, O.; Bocklitz, T.; Rösch, P.; Popp, J. Detection of multi-resistant clinical strains of E. coli with Raman spectroscopy. Anal. Bioanal. Chem. 2022, 414, 1481–1492.
38. Shen, H.; Rösch, P.; Pletz, M.W.; Popp, J. In Vitro Fiber-Probe-Based Identification of Pathogens in Biofilms by Raman Spectroscopy. Anal. Chem. 2022, 94, 5375–5381.
39. Shen, H.; Rösch, P.; Popp, J. Fiber Probe-Based Raman Spectroscopic Identification of Pathogenic Infection Microorganisms on Agar Plates. Anal. Chem. 2022, 94, 4635–4642.
40. Rodríguez-Lorenzo, L.; Garrido-Maestu, A.; Bhunia, A.K.; Espiña, B.; Prado, M.; Diéguez, L.; Abalde-Cela, S. Gold Nanostars for the Detection of Foodborne Pathogens via Surface-Enhanced Raman Scattering Combined with Microfluidics. ACS Appl. Nano Mater. 2019, 2, 6081–6086.
41. Bai, X.; Shen, A.; Hu, J. A sensitive SERS-based sandwich immunoassay platform for simultaneous multiple detection of foodborne pathogens without interference. Anal. Methods 2020, 12, 4885–4891.