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A BIOANALYTICAL APPROACH USING SURFACE-ENHANCED RAMAN SPECTROSCOPY

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ABSTRACT

Surface-enhanced Molecular adsorbed on rough metal surfaces enhance Raman scattering via Raman scattering (SERS), a surface-sensitive method. 107 -1015 is the maximum enhancement factor possible, making this method sensitive enough to detect single molecules. The use of SERS-tags has evolved into clinical diagnostics; the enhancement of the intrinsic signal of biomolecules on SERS active materials shows tremendous promise for the analysis of biomolecules and potential biomedical assays. To date, the detection of the de novo signal has been reported for a wide variety of biomolecules. Various classes of biomolecules are examined in this review for the signals they have been observed and the experimental details that enable their detection. We have found.

Keywords: Raman spectroscopy, surface-enhanced Raman scattering, biological simulates, SERS, bioanalytical

INTRODUCTION

Raman spectroscopy is concerned with radiation scattering from a sample. Scattering occurs when an incident photon interacts with the electric dipole of a molecule. Resonance Raman spectroscopy (RRS) involves the use of laser excitation light whose energy coincides with an electronic transition in the target molecule. This approach improves the

scattering cross-section and selectively enhances (by orders of magnitude) certain spectral features. 3 The resonance wavelength depends on the analyte of interest; for example, excitation at 400 nm coincides with a resonant enhancement of the Soret band of haemoglobin (the major constituent of RBCs). Resonance Raman spectroscopy has previously been reviewed elsewhere. Plectroscopy has been discussed in the past. Another common type of Raman spectroscopy enhancement is surface-enhanced Raman spectroscopy (SERS), which was first reported in the 1970s. 4, 5 Through the use of metallic surfaces or plasmonic resonance-capable nanoparticles, surface-enhanced Raman spectroscopy boosts Raman scattering. [1]

Microorganisms are ubiquitous in the environment and found in and on mammalian hosts in a complex systematic relationship, in these environments these organisms occur predominantly as complex multi-organism biofilms. The microbial world forms a huge family of organisms that exhibit the greatest phylogenetic diversity on Earth and thus colonise virtually our entire planet. Due to this diversity and subsequent complex interactions, the vast majority of microorganisms such as Pseudomonas putida and Azotobacter chroococcum are directly involved in a huge number of natural bioprocesses which contribute a central role toward the homeostasis of life on Earth, whilst a small and important minority which includes Vibrio cholerae, Campylobacter, and Mycobacterium tuberculosis is responsible for various infectious diseases. [2]

In SERS-based detection schemes, four main concepts are known: (1) label-free or direct SERS sensing is mostly applied and relies on the high affinity of the target analyte towards the metallic surface allowing for a SERS-based detection even in complex matrices. As analytes, mostly low molecular weight substances, e.g. drugs, metabolites, organic pollutants or small biomolecules, are targeted by label-free SERS approaches in complex matrices such as biofluids or surface water sources. (2) In a further sensing strategy, SERS tags which are composed of plasmonic nanoparticles (NPs), Raman reporter molecules and recognition elements are applied as stable and bright labels in e.g. DNA detection schemes or immunoassays. [3]

It is well known that many life activities in cells are accompanied by changes in pH, so local detection of pH in cells is important to understand the mechanism of their activities. Mitochondrial respiration and fermentation metabolism produce large amounts of CO2 and lactic acid, respectively. All tissues produce acid, including tumors. Compared with other ions, the detection and quantification of hydrogen ions in living cells are of more concern in the field of biosensors at present, because they play a crucial role in the physiological and pathological processes of single cells and organisms, and the change of their

concentrations directly affects the physiological functions of normal living organisms. [4]

Raman spectroscopy is very useful in drug analysis due to advantages such as ease of use, minimal sample handling, and the significant differences in scattering strength between packaging materials, tablet excipients, and active drug components. It can also be used to identify isomers and to determine energy difference between isomers. These advantages, in combination with fibre optics and microscopes, have enabled the use of Raman spectroscopy as a quality control tool in the pharmaceutical industry. One major disadvantage with conventional Raman spectroscopy is the small scattering cross section of many materials. Surface enhanced Raman scattering has been widely used in many fields due to its unique advantages such as high sensitivity, strong specificity, good reuse ability and optical stability, especially in biological detection and biological imaging in recent years. [5]

LITERATURE REVIEW

Jialong Sun, Wei Li, Xuerui Zhu, Saisai Jiao, Yunwei Chang, Siwei Wang, Shijie Dai, Ruimin Xu, Menghua Dou, Qianjin Li, Jianlin Li. (2021) In order to compete for binding antibody-functionalized SERS nanotags, a competitive SERS immunoassay was developed using antigen-modified SPCMs and mycotoxins. This showed broad linear detection ranges of 0.01–10 ng/mL for ochratoxin A (OTA) and 0.01–0.1 ng/mL for zearalenone (ZEN) as well as low detection limits of 0.82 pg/mL for AFB1, 1.43 pg/ The method's recovery rates for the three mycotoxins were found to be between 70.35 and 118.04 percent in the spiked cereal samples, which was in line with the results of the standard enzyme-linked immunosorbent assay. The SERS immunoassay for mycotoxin detection also showed high specificity and good repeatability and reproducibility. With the new microsphere-based SERS immunoassay biochip, there is only one step of reaction required, and fluorescence and chemiluminescence background signals are no longer a hindrance. SERS-based microsphere suspension arrays for new targets are now possible thanks to this research. [6]

Damin Liu, Xiaoyu Song, Wencai Yi, Yahui Li, Qinghong Kong, Hua Bai, Mingqiang Zou, Guangcheng Xi. (2021) Single-crystal porous WN, Mo2, and V2N with strong surface plasmon resonance, photothermal conversion, and surface-enhanced Raman scattering effects are prepared for the first time by this method for these new materials. As new concept absorbing media, hydrated metal oxides and metallic metal oxides have a remarkable high-temperature microwave heating effect and play important roles in the formation of TMNs, in contrast to conventional low-temperature microwave absorbing

media such as water and polymers. There is a new microwave method for preparing high-lattice energy compounds with a high specific surface area that has just come out of research. [7]

Kuo Yang, Kai Zhu, Yuanzhe Wang, Ziting Qian, Yizhi Zhang, Zhaoyan Yang, Zhuyuan Wang, Lei Wu, ShenfeiZong, Yiping Cui (2021) This sensor has a universally high adsorption efficiency for various gases due to the use of MXene, while the generation in situ of gas vortices in the sophisticated nanomicro structure increases residence time of the molecule inside of the SERS-active area, increasing sensitivity. In the proof-of-concept experiment, a limit of detection (LOD) of 10–50 ppb was achieved for three typical volatile organic compounds (VOCs) according to the intrinsic SERS signals of gas molecules. The periodic 3D structure also solves the SERS substrates' general repeatability issue. In addition, a 90.6 percent accurate classic least-square analysis (CLS) revealed the exact composition of the gas mixture. In addition, a chromatic barcode based on CLS results was developed to visually read out complex sample compositions. [8]

JaciaraBär, Anerise de Barros, Davi H. S. de Camargo, Mariane P. Pereira, Leandro Merces, Flavio Makoto Shimizu, Fernando A. Sigoli, Carlos César BofBufon, Italo Odone Mazali (2021) It is still a hot subject in the SERS area to look into the consequences of increased Raman signals and to prepare high-quality, dependable SERS substrates. We describe an impact on gold nanorods (AuNRs) as a SERS substrate based on shape-induced increased Raman scattering (SIERS). Raman experiments using incoming radiationproduce spatially dispersed interference patterns on bare V-shaped Si surfaces, resulting in constructive interference that enhances the Raman signal. A 4.29 increase in Raman signal intensity has been seen in experiments using V-shaped Si microchannels as opposed to flat Si substrates. Detection at ultra-low concentrations is made possible by combining V- shaped micro channels with homogeneous AuNR aggregates. This enables reproducible SERS substrates with excellent performance and sensitivity. [9]

Cheng Zong, Mengxi Xu, Li-Jia Xu, Ting Wei, Xin Ma, Xiao-Shan Zheng, Ren Hu, and Bin Ren (2018) This study offers an in-depth look into bioanalytical SERS, with particular attention paid to the problem of dependability. We begin by introducing the SERS mechanism in order to assist in the development of robust SERS experiments capable of yielding accurate results. In order to develop functionalized SERS nanoparticles for target detection, we present the current knowledge of the interaction between nanomaterials and biological systems, namely live cells. For systems from biomolecules to pathogens to live cells, we present the current state of label-free (direct) and labelled (indirect) SERS detection and address the possible interferences from experimental design, measurement

settings and data processing. Ending with a perspective on the main difficulties in bioanalytical SERS, including as reproducibility and sensitivity, as well as spatio-temporal resolution. [10]

SURFACE-ENHANCED RAMAN SCATTERING DETECTION OF BIOLOGICAL AGENT SIMULANTS

Nutritional and biochemical assays are often used to identify pathogenic bacteria. Despite the fact that these methods may provide results quickly, they are costly and require the use of highly trained people. As a supplementary whole-organism fingerprinting method for the fast characterization of bacteria, SERS has recently been proven to have tremendous promise as of late. Initial biological agent simulant SERS measurements on silver oxide thin films failed (data not shown). However, just 15 minutes of UV lamp illumination yielded a SERS signal (Figure 1) Although these SERS spectra may provide structural information; it is challenging to analyze them.

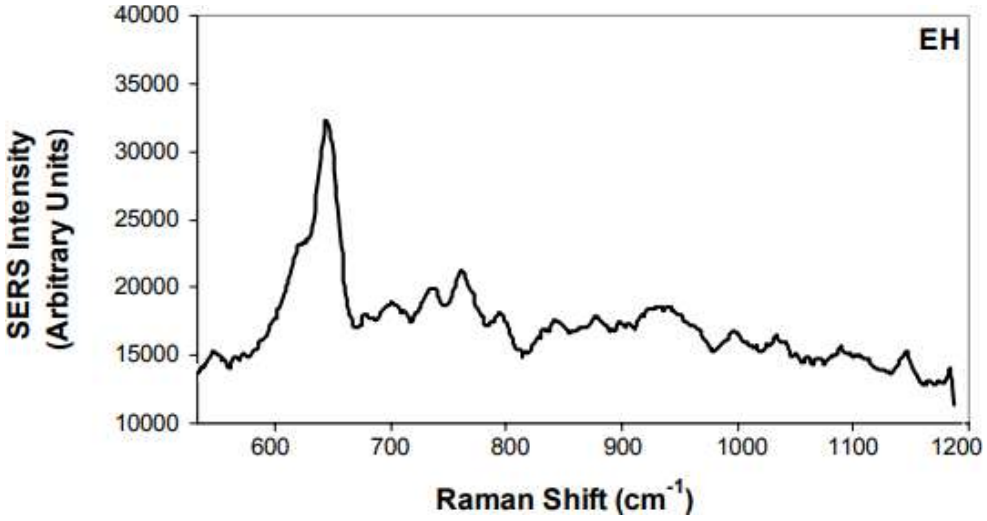

Figure 1 SERS spectrum of erwiniaherbicola on Ag silver oxide film grown on a glass slide after UV illumination for 15 min.

It's possible that biochemically complicated samples like bacteria have a plethora of SERS-active vibrational modes present. Using a priori biochemical knowledge, it is feasible to focus research on certain peaks; nevertheless, any peak designations may only be considered speculative at best. Despite the fact that many scientists have looked into using SERS to characterize bacteria, only a small number of studies have demonstrated that the SERS spectra of different bacteria can be distinguished. Although SERS has the ability to generate spectra useful for fast whole-organism fingerprinting, new research shows that such hyperspectral data may be utilized to distinguish different microbes down to the strain level using simple cluster analysis. There are similar research activities in our organization now and the results will be published elsewhere.

INSTRUMENTAL CONSIDERATIONS

When using SERS probes for bioanalysis, it's critical to keep Raman instrumentation in mind as well. An assay's "optimal instrument" depends on the SERS probes, sample format, and quantity and kind of information needed. When a probe is chosen, the first thing to consider is the wavelength of the laser. Raman spectroscopy's capacity to measure with a single or many laser excitation wavelengths is one of its numerous advantages. When using SERS probes, however, this decision may be made more difficult since the optimum laser excitation wavelength depends on the particle plasmon resonance and resonant contributions from Raman reporter dyes. The existence of auto-fluorescence from biological components and low tissue penetration depth of visible excitation wavelengthsfurther complicate this problem. Accordingly, many SERS experiments are currently being conducted using near-IR (NIR) wavelength illumination, which may assist to reduce fluorescence and improve tissue penetration in future applications.

INTERPRETING THE DATA

All analytical chemistry methods, including this one, must take data analysis into account. SERS probes are similar to fluorescence in that they may respond to a binding contact with a "on" or "off" type response. If a signal's intensity response is calibrated against known or independently observed values, this may be quantifiable. Univariate analysis is shown here. When analyzing SERS data, multivariate chemometric approaches are often used, including more advanced chemometric techniques. While using SERS probes to detect biochemical signals from native molecules, the spectra generated will contain information on a wide range of biological species that were present near the nanoparticle surface. By generating principal components that explain the most variance in the data, principle component analysis (PCA) is often used to decrease data dimensionality. The partial least squares regression (PLSR) method is a multivariate supervised analytic technique that models the spectral response to incremental experimental changes that are known. Individual contributions to multiplex spectra may now be identified and measured thanks to this advancement in multiplex SERS probe capabilities. In a multiplex test, a method known as direct classical least squares (DCLS) analysis is frequently used to differentiate between the contributions of various SERS probes. SERS spectra have more multiplexing potential than univariate fluorescence analysis because of the many distinct peaks in the spectra that provide multivariate data.

Point-of-care (PoC) solutions based on SERS for infection diagnosis and targeted treatment

DNA and antibody arrays may now be quickly tested using new high-throughput diagnostic

techniques. SERS is a good option for large-scale clinical sample screening because of the decreased assay cost and time, as well as the extended storage practicality. Sequencing DNA may be accomplished using SERS without using fluorescent markers.

Antibiotic multiresistance (AMR) is still evaluated via plating and expensive susceptibility testing in time periods ranging from hours to days. It's possible to identify the genes that cause multiresistance using specialized molecular biology methods, but they come at a high cost in terms of time and effort. SERS has shown encouraging outcomes in the therapeutic setting when used for spectrum pathology or even for quickly identifying a focused therapy. Antibiotic susceptibility research is critical for combating overuse and abuse of antibiotics, as well as dealing with the growing problem of drug resistance. Without labelling or any other kind of sample preparation, real-time pathogen susceptibility measurements would be ideal. In recent years, researchers have been combining DEP–Raman spectroscopy in an attempt to find the most straightforward, reliable and efficient method.

With the use of bacteria-aptamer@Ag NPs, a new fast and sensitive SERS-based AST method has recently been developed. When antibiotic doses below the MIC were used, the representative pathogens exhibited a similar spectral profile, with a rise in the 735 cm^{-1} SERS band after an hour. The minimum inhibitory concentration (MIC) was found in around an hour. A very sensitive in situ fingerprinting technique, SERS may be used to evaluate antibiotic susceptibility on both Gram-positive and Gram-negative bacteria by using properly chosen aptamers. As a result of analyzing the evolution of the strongest SERS band (735 cm^{-1}) in relation to antibiotic concentration, this SERS–AST approach proved to be a quick and reliable method of detecting live bacteria while also monitoring how sub-MIC antibiotic pressure leads to increasing bacteria colony-forming units, as demonstrated (CFUs).In addition, Bauer et al. recently published on a Raman-based method for reliable and accurate assessment of bacterial pathogen antibiotic susceptibility. The sensitivity of Raman-based antibiotic susceptibility testing may be further enhanced by using stable isotope labelling. Within three and a half hours, a procedure for Raman-based AST was evaluated for Gram-positive and Gram-negative bacteria, including sample preparation, measurements, and analysis. The NPs' surface is where bacteria first come into contact with them. By adhering to biological binding sites, silver nanoparticles release silver ions, which adhere to biological binding sites and have an antimicrobial effect. However, nothing is known about the relationship between bacteria's surface characteristics and the antibacterial processes of nanosilver/nanomaterial. Silver-doped laser-induced graphene-coated surfaces have recently been shown to resist biofilm

development, with applications in rural water source cleaning and catastrophe scenarios. In situ methods seem to have a limited ability to study interfacial interaction phenomena in depth so far. To learn more about the interactions between silver nanoparticles and bacterial cell walls, researchers are using in situ SERS analysis and barcoding to identify the origin of the observed SERS bands and their precise assignments.

Despite the fact that SERS may be considered as a very sensitive detection method, particularly in the case of biological materials, the repeatability of SERS spectra, while conducting single-cell SERS observations, is still poor. The nanoparticles may either coat the bacterial cell wall or be directed into the bacterial cell when preparing samples for SERS bacterial detection utilizing colloids as active substrates. Cell wall components provide the SERS spectral information in the first instance, whereas the cytoplasm is probed in the second. As a result, SERS spectra may include information from both within and outside the cell, increasing their specificity. Molecular specificity is carried by the chemical and biological components of the bacterial outer membrane and pertains to the strain, growth phase, and metabolic state. SERS barcoding model system can detect and identify "blind" actual samples even in mixes using biochemical information from seven strains of V. parahaemolyticus.

The interpretation of SERS spectra is not simple. To prevent spectrum variations due to drying, try utilizing micro channels (micro capillaries) as a detection window, or record spectra via a water layer to shield the sensor from the drying process. It is also important to obtain as much spectrum information as possible from bacterial biomass by performing purification or selective preconcentration procedures (without contributions from the matrix of EPS species). SERS signals are often related to cellular wall components including flavin derivatives and polysaccharides.

The next section discusses several methods for circumventing the limitations of single-bacterium SERS detection. A major difficulty of SERS-based assays for detecting and analyzing bacteria is to develop and produce such methods that are capable of collecting bacteria from water, phlegm, saliva or blood samples and to directly load them onto SERS substrates without affecting their SERS-sensing capacity.

In vitro measurements

For illness diagnosis and subsequent treatment, there is a critical need to create non-invasive and non-destructive techniques. Biomarkers can be detected in vitro utilizing SERS since it's fast and sensitive while also being able to identify several targets at once.

It is important to conduct in vitro research in order to establish the groundwork for future in vivo experiments that may be successful, however not all in vitro studies are intended to be moved into a living environment.

CONCLUSION

To sum it up, SERS-activated systems provide excellent bioanalytical sensitivity as well as potential multiplexing capabilities. Many different SERS-based assays, including those using labels and others that don't, may be used in biomedical and clinical settings due to the variety of microfluidics chips, paper-based substrates, and so on. More potential uses emerge like high-throughput tests and next-generation surgical therapy when SERS multiplex detection capability is combined with in vivo endoscopic methods. The examples of seminal SERS probes used in bioanalytical assays are summarized in this study. Research in this area is much more advanced now than when SERS was discovered in 1974. The considerable benefits it can provide in terms of fast, sensitive, and multiplexed measurements have led to its subsequent utilization for biological sample analysis.

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