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SERS-Based Biosensors Combined with Machine Learning for Medical Application**

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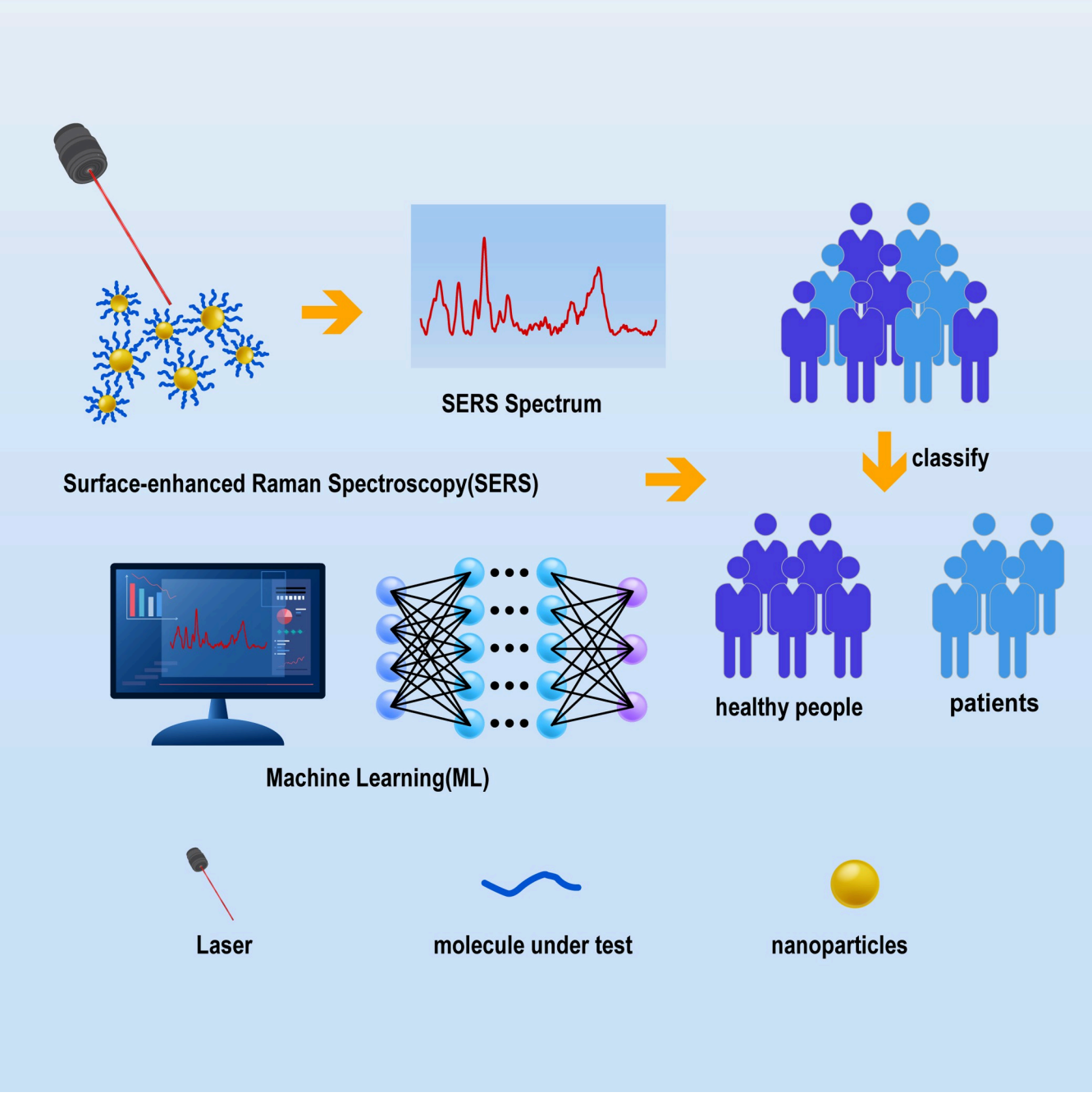

Surface-enhanced Raman spectroscopy (SERS) has shown strength in non-invasive, rapid, trace analysis and has been used in many fields in medicine. Machine learning (ML) is an algorithm that can imitate human learning styles and structure existing content with the knowledge to effectively improve learning efficiency. Integrating SERS and ML can have a promising future in the medical field. In this review, we summarize the applications of SERS combined with ML in recent years, such as the recognition of biological molecules, rapid diagnosis of diseases, developing of new immunoassay techniques, and enhancing SERS capabilities in semi-quantitative measurements. Ultimately, the possible opportunities and challenges of combining SERS with ML are addressed.

1. Introduction

Biological diagnostics are often used clinically as part of the disease diagnosis, but traditional methods of analysis have the disadvantages of being time-consuming, expensive and high demanding on laboratory researchers, which makes the development of a new rapid, inexpensive and immediate test of great clinical importance.[1,2]

Raman spectroscopy is a molecular vibration-based method applied in studying molecular structures. In 1974, the surface-enhanced Raman spectroscopy (SERS) effect was first discovered by Fleischmann et al. and it was found that the intensity and frequency of the bands are increased compared with Raman spectroscopy.[3] In the case of plasma nanostructure aggregation, large field enhancements can be observed in the slits between nanoparticles, which we call "hot spots", where the SERS signal is greatly enhanced and characteristic fingerprint peaks of the target material are obtained.[4–6] Researchers have created "hot spots" for SERS by preparing different nanoporous surface materials to enable more sensitive molecular detection. The Raman signal reflects the fingerprint information of a molecule, while SERS makes the fingerprint information of a molecule more concrete and precise. By monitoring the SERS signal, we can make a preliminary determination of the presence of the target molecule, the structure of the substance and the process by which the reaction took place.[7–9] In recent years, SERS has increasingly been viewed as one of the most vital research methods in the field of single-molecule science, and as SERS becomes more widely used, there is a high expectation for *in vivo* imaging, rapid diagnosis of diseases, and rapid detection of trace substances.[10–13] Recognizing this, there are a growing number of auxiliary means designed to improve the depth and speed of analysis. For instance, changing SERS substrates is a significant factor to change the enhancement factor.[14–17] Alternatively, exchanging the carrier for target molecules fulfills the multiplexed, molecular sensing and imaging simply and cost-effectively.[18,19] These methodologies are beneficial for the rapid and trace detection of target substances under non-invasive conditions and can boost the intensity of SERS spectra under certain conditions, which is highly effective for obtaining good spectral profiles.

It is always pursued to be more efficient and accurate in analyzing Raman spectra after obtaining more accurate Raman spectra. ML is an algorithmic technique that can handle large, complex, and different types of data, including such types as Support vector machine (SVM), Neural networks (NN), Principal component analysis (PCA), Partial least square (PLS), deep learning, and decision tree learning. Such algorithms can be applied to many aspects of healthcare, from diagnosis and treatment to individualized drug selection. ML is expected to be able to specify accurate medical solutions like excellent medical doctors.[20–24]

SERS, when combined with ML algorithms, can be analyzed with a higher degree of accuracy. Thus, the limitations imposed by complex data can be addressed. The way we will introduce has been proved to be highly effective because of the ability to calculate SERS with quantum mechanical accuracy which is greatly significant in thousands of molecular dynamics conformations.[25] With the rapid development of SERS and ML, its application is getting wider and wider in medicine.

Because both SERS and ML have a wide range of applications in their respective fields, the combination of machine learning and SERS has significant advantages. Therefore, many research papers and reviews which combine ML with SERS have been published in recent years. Some of these reviews discuss their use in medical diagnostics and treatments,[26,27] others describe machine learning driving the development of sensors,[28,29] and others address their use in DNA, protein and drug detection.[30] However, there is a lack of comprehensive reviews on the use of ML in conjunction with SERS in medicine.

The aim of this review is to fill a necessary gap in the literature regarding the application of ML combined with SERS in medicine and to provide a comprehensive overview of articles published since 2015. Such reviews are essential for the field to move forward.

Our review will present the application of SERS and how ML has been used in conjunction with SERS applied in disease classification, diagnosis of infectious diseases and cancer, detection of metabolites *in vivo*, and the creation of medically

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[**] *SERS = Surface-enhanced Raman spectroscopy*

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relevant databases. Finally, possible opportunities and challenges of combining SERS with ML are addressed.

2. The Way Machine Learning Works

Machine learning is the part of artificial intelligence that performs tasks that need to be done in a data-driven way.[31] The fact that it works in a data-driven way means that it can learn from the data and automatically improve the predictive model, and that the predictive model becomes more accurate as more data is fed in. Machine learning can extract data features from raw data to obtain knowledge, and use this acquired knowledge to make decisions to solve complex practical problems. For example, machine learning models can be used to diagnose or classify diseases by learning from a large number of scanned images.[32–34]

The concept of machine learning can be traced back to Turing's 'machine learning' in the 1950s and the development of the first neural network, which focused primarily on military experiments. The opening of the Artificial Intelligence in Medicine (AIME) conference in 1985 saw growing optimism about the use of machine learning in medicine, and a series of creative studies emerged. Since the 2010s, deep learning has entered another phase of rapid development (Figure 1).[35]

Traditional methods of data analysis in medicine are mainly based on the testing of causal hypotheses and the selection of models around the significance and in-sample goodness of fit. While machine learning focuses more on the predictive performance of models in the mathematical dimension and the generalizability of model generalization. It uses a subset of the relevant characteristics of variables in different formats for model creation and uses pattern analysis and dimensionality reduction methods to train a model that best fits the characteristics of the corresponding variables.[36,37] For precise recognition, it is necessary to establish a large reference sample library with uniqueness and accuracy for each sample in the sample library when performing machine learning. Machine learning can learn data from a large amount of data to build a model and improve the accuracy of the models as the learning time increases, or experienced researchers can select analytical models that correspond to the problem, thus solving data processing where classical linear methods are inadequate(Figure 2).[38,39] After acquiring a large amount of data, it is also necessary to classify the data into a train set and a test set. The train set is the data set for developing the machine learning

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Feng Chen received his B.M.S. in Xi'an Jiaotong University in 2005 and his Ph.D. in Forensic Medicine from Xi'an Jiaotong University in 2011. Afterwards, he undertook two years of postdoctoral studies at Medical College of Georgia, Augusta, Georgia. He was appointed as a professor and director at the department of forensic medicine at Nanjing Medical University in 2013. His main research expertise involves cardiovascular disease and sudden cardiac death, molecular genetics and molecular biology.

Yan Ding obtained her undergraduate degree from Xuzhou Medical University and her master's degree from the Department of Forensic Medicine, School of Basic Medical Sciences, Nanjing Medical University. Her current research interest is focusing on constructing novel SERS biosensors and the application of SERS and machine learning in medicine.

Yang Sun received her B.M.S. in the Department of Forensic Medicine, Tongji Medical College of Huazhong University of Science and Technology in 2020. Now she is studying for a Master Degree in the Department of Forensic Medicine, Nanjing Medical University. Her postgraduate research direction is the application of flexible SERS sensors and electrochemical sensors in forensic toxicology analysis.

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Qiao-Yan Jiang received her Master's degree in Nanjing Medical University. She is currently working in the Pathology Department of Taizhou Central Hospital(Taizhou University Hospital). Her research focuses on the application of new nanomaterial SERS sensors and electrochemical sensors in forensic toxicology analysis and clinicopathological diagnosis.

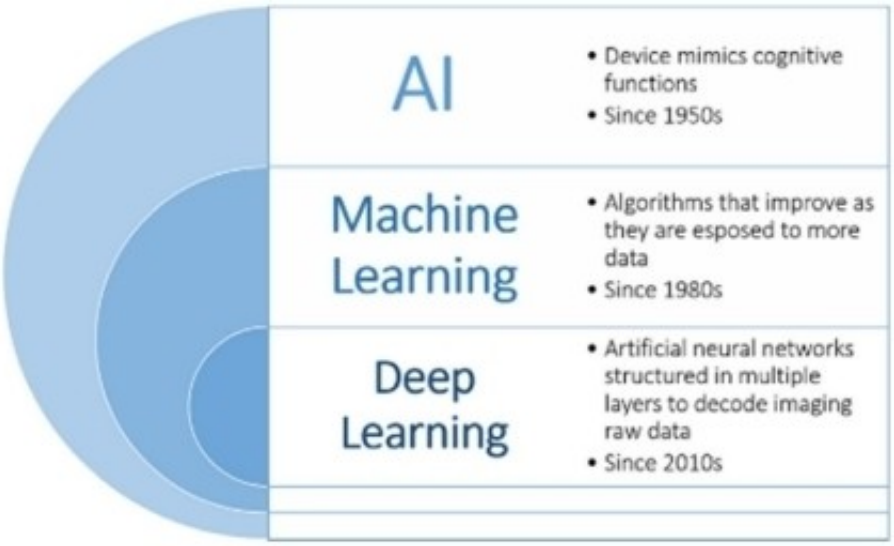

Figure 1. Infographic depicting deep learning as a subset of artificial intelligence. Reproduced from Ref. [34] Copyright (2022), with permission from Elsevier.

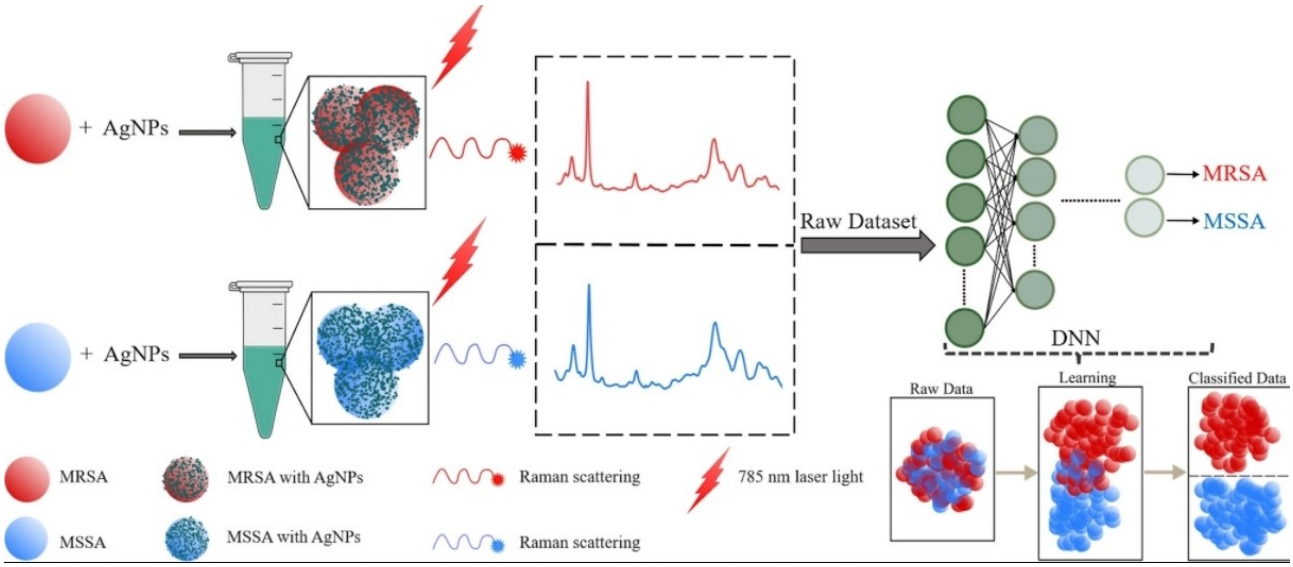

Figure 2. General workflow of deep learning-based spectral data analysis for the discrimination of antibiotic- resistant bacteria. Reproduced from Ref. [39]. Copyright (2021), with permission from Springer Nature.

model and the test set is used to evaluate the resulting machine learning model after the model has been trained.[40]

Machine learning encompasses very specific chemometric methods, each of which can solve different practical problems. Two of the main categories of machine learning are supervised learning and unsupervised learning. Supervised learning is to find the optimal algorithmic model based on an existing dataset when the relationship between the input data and the output data is clear. Whereas unsupervised learning is where we do not know the relationship between the data in the set, the features, but have to get the relationship between the data based on a certain model or clustering.[41]

Several machine learning algorithms are used to analyze Raman spectra, including many different sub-methods such as principal component analysis (PCA), support random vector machine (SVM), convolutional neural network (CNN), distributed arithmetic (DA), Quadratic Discriminant Analysis (QDA), linear discriminant analysis (LDA), partial least squares (PLS-DA), artificial neural network (ANN), random forest (RF) and other algorithms. Table 1 provides a brief description of the main methods discussed in this paper, and the reader is referred to other more detailed literature for detailed merits and demerits of each method.[42–45]

Table 1. Brief description of the main methods discussed in this paper.

Name	Function	Description
PCA	dimensionality reduction	PCA simplifies the data set by converting multiple indicators into a few composite indicators
SVM	Binary classification of data	SVM performs non-linear classification by kernel method
CNN	Representation learning	CNNs are feed-forward neural networks that include convolutional computation and have a deep structure
ANN	Simulation of neurons	ANN is an information processing system that mimics the structure and function of the brain's neural networks
DA	Global Optimization Search	DA uses 5 formulas to determine the optimal algorithm
QDA	Discriminant analysis	QDA determines which of the known types the predicted item belongs to
PLS	Minimisation of errors	PLS is a mathematical optimization technique that allows for a minimalist approach to value
LDA	Dimensionality reduction	LDA clusters the data to make the different classes of data more discrete
RF	Decision Trees	The class of the decision tree output in a random forest is determined by the plurality of the classes of the individual tree outputs

3. The Application of SERS Combined with Machine Learning in Medicine

Since machine learning offers the ability to learn from examples, it is not a stretch to say machine learning can be applied in prognosis, diagnosis, and treatment, which values accurate and rapid techniques.[46] With the combination of machine learning and SERS, further use of trace substances or small molecules can be utilized to predict diseases or classify diseases, bacteria and biomarkers. The strength of machine learning is its ability to distinguish between two or more substances rapidly and accurately that are so similar in the SERS spectra that the human eye has difficulty distinguishing them.

3.1. The application in classifying biomolecules

Like many bacteria, viruses or DNAs are functionally or structurally similar. The development of new rapid detection methods is necessary because of the complex steps and high demands placed on the laboratory personnel to carry out the analysis using traditional methods.[47,48]

When classifying and identifying biomolecules, PCA methods are often used to reduce the dimensionality of complex spectral data and simplify the resulting spectral data. The selection of machine learning algorithms is then carried out, with DNN and SVM being the common means of binary identification. Alternatively, PCA can be used directly to identify feature peaks that differ in the main components of the SERS spectra. Combining the two machine learning algorithms sometimes results in better classification results.

3.1.1. The application in classifying in bacteria

Bacteria are often a common cause of infection and inflammation, and antimicrobial drugs are commonly applied as a treatment for the diseases that result from bacterial infections. Nevertheless, as the misuse of antimicrobials has led to the growth of multidrug-resistant strains, it is essential to accurately identify the sequence types of the different multidrug-resistant strains for optimal disease control and infection prevention strategies. Meanwhile, accurate identification of the type of bacteria causing the disease is the most important means of prescribing line-specific antibacterial drugs.[49,50] Genotyped antibiotic susceptibility testing (AST) requires complete and adequate training of the testers to identify resistance genes which are truly capable of expressing resistance.[51]

Combining machine learning with SERS allows for more reliable analysis and identification of different resistance genes and the corresponding bacteria. The PCA algorithm can reduce the dimensionality of complex SERS spectral data and simplify the content of the dataset. Combined with the SVM algorithm which can perform a better binary classification of the dataset, these algorithms can solve many classification problems in medicine. Measurement of SERS with direct gold nanoparticle deposition as a substrate for *E. coli* ATCC25922 (control), *E. coli* ST131:O75 and *E. coli* ST1193:O25 in blood yielded three similar SERS spectra. During the measurement, the ring area of the coffee swap effect is selected as the ideal location for detection to obtain the best SERS enhancement and Raman signal. It is a challenge to define the type of *E. coli* measured by operator judgement. However, SVM-assisted PCA scoring plots showed excellent performance in classifying these three types of strains.[52]

Deep learning networks (DNNs) are self-learning and self-algorithm selection and training by extracting features from input data. It is a popular branch of machine learning in recent years and is suitable for databases with large amounts of data as well as image analysis. Aydin et al. employed a deep neural network (DNN) with stacked autoencoders (SAE) on methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA), which eliminates complex data preprocessing steps, and achieved an accuracy of 97.66% and an AUC of 0.99%.[39] By SERS signals obtained by mixing gold and silver nanoparticles, Wang et al. compared three unsupervised machine learning methods and ten supervised learning methods to classify the SERS spectra of 2752 staphylococci, respectively, and the final results showed that convolutional neural network (CNN) which is a branch of DNN was the best model for predicting Staphylococcus species.[53] Wang and his team used eight supervised learning methods to classify and predict carbapenem-sensitive Klebsiella pneumoniae (CSKP) and carbapenem-resistant *Klebsiella pneumoniae* (CRKP), respec-

tively, with the CNN algorithm performing the best out of the eight.[54] Other researchers used a wide range of machine learning algorithms to discriminate between 15 different genera of bacterial pathogens in clinical settings, and ultimately found that CNNs can accurately predict pathogen species with good noise immunity. It can be shown that the CNN has good performance in differentiating bacterial spectra.[55]

Zhao and his group utilized AgNR arrays deposited as SERS substrates to obtain Raman signals and used RamanNet, a deep learning algorithm designed for Raman spectroscopy data analysis, to classify bacterial endotoxins, and the classifier showed 100% accuracy. The emergence of RamanNet has also given a strong impetus to the use of machine learning in Raman data analysis.[56]

3.1.2. The application in identifying viruses

For SERS, modification of the substrate with a high binding capacity receptor corresponding to a specific target substance can improve the capture efficiency of the SERS signal of the target substance.[57] The SERS spectra obtained in this way can be more easily classified.[58]

PCA methods are widely used in the field of virus detection as well. Luo et al. developed a 'virus trap' composed of gold nanoneedles array modified with a functional host cell receptor angiotensin converting enzyme 2 of coronavirus spinous process protein (S protein) to capture the SERS signal of COVID-19. After obtaining the SERS signal, PCA was used to obtain the principal component coefficient in the spectrum. Then discriminant analysis (DA) is used to classify unknown spectral samples. The LOD of this method is as low as 80 mL^{-1}(Figure 3).[59] Li et al. also applied the PCA algorithm to classify the serum and saliva of three different viruses, including COVID-19, HAdV and H1 N1, and the classifier showed good classification ability.[60] Pan used the PCA-SVM method for the rapid detection of COVID-19 as well.[61]

3.1.3. The application in classifying DNAs

When identifying and classifying different DNAs, it mainly relies on four bases with different relative contents in DNA to distinguish between different DNAs.[62] He et al. used silver nanoparticle-modified in silico to capture signals from different DNAs and trained the data with a very popular way called Deep Neural Network (DNN) to find the most suitable model for data analysis model. Selective identification of DNA targets with the resulting model achieved an accuracy of 90.28%.[63] J. Vikesland and co-workers used the method of slippery liquid infused porous SERS for label-free detection of ssDNA and constructed

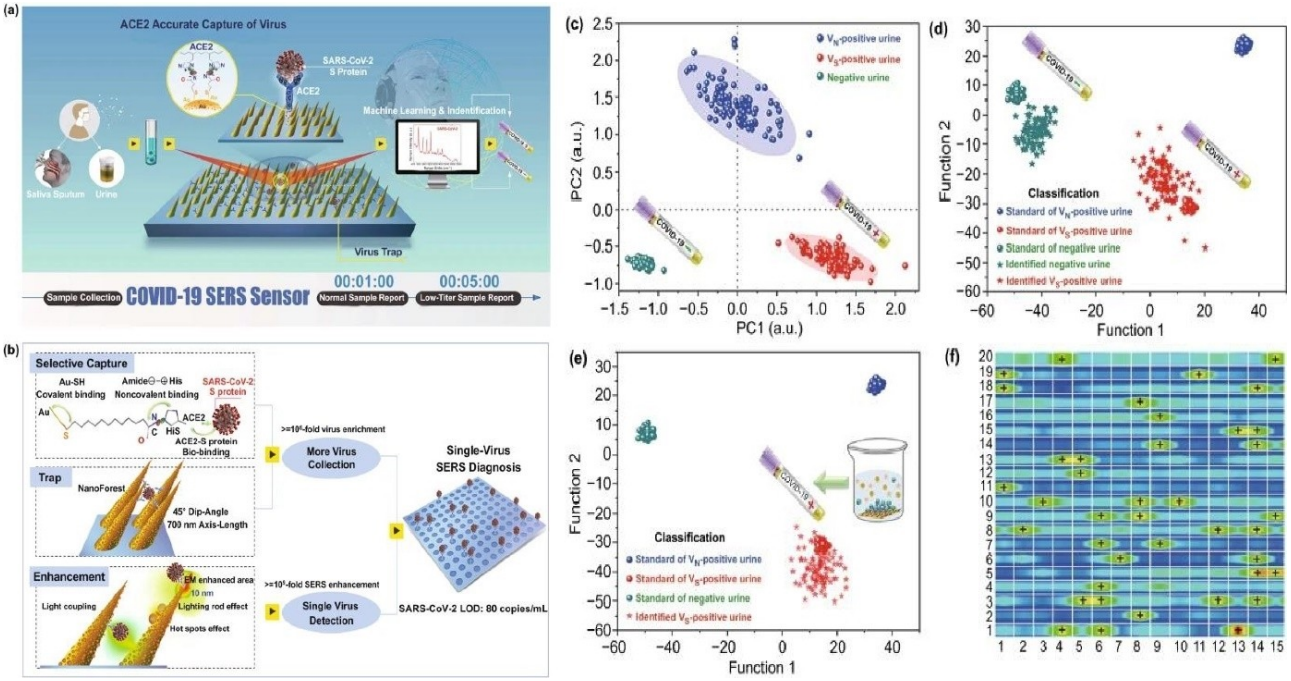

Figure 3. Schematic diagram of COVID-19 SERS sensor design and single-virus detection mechanism. (a) Schematic diagram of COVID-19 SERS sensor design and operation procedure. (b) Schematic diagrams of single-virus detection by selectively capturing and trapping virus, and the multi-SERS enhancement mechanism. (c) Key features of SERS patterns to classify the urine samples infected by VS (simulated contaminated water by SARS-CoV-2 virus), VN and healthy people via PCA. (d) DA results to identify the urines for chronic nephritis and VS-containing chronic nephritis. The green, red, blue balls represent the standard of negative urine, the standard of VS-positive urine, the standard of VN-positive urine. The green and red stars represent identified negative urine and identified VS-positive urine. (e) DA results to identify VS and VN virus mixed in the adult's urine (2200 copies mL^{-1}). The green, red, blue balls represent the standard of VS-negative urine, the standard of VS-positive urine, the standard of VN-positive urine. The red star represents identified VS-positive urine. (f) SERS mapping (40×30 μm^2) of 300 measuring area for one urine sample, 42 dot-measuring-area can be identified as VS-positive. Reproduced from Ref. [59], Copyright (2021), with permission from Springer Nature.

a Tr-SVM classifier to distinguish 12 different gene sequences, with an accuracy of 90%.[64]

3.1.4. The application in identifying extracellular vesicles

Extracellular vesicles are a collective term for vesicular structures released by cells with various membrane structures, which have different subpopulations and function to communicate with cells. Nevertheless, extracellular vesicles are difficult to detect and characterize because of their small size.[65,66]

Langugne-Labarthet and co-workers applied electron-beam lithography (EBL) to fabricate plasma-activated gold nanopore arrays of different sizes and shapes that can be used to capture the SERS signal of specific extracellular vesicles depending on size. The authors captured SERS spectra of extracellular vesicles originating from pancreatic tissue (Panc-MSC) and bone marrow (BM-MSC) and used principal component analysis (PCA) to identify characteristic peaks of major component differences, finally using logistic regression to achieve 89% accuracy, 89% sensitivity and 88% specificity.[67] Tian et al. used polyethylene glycol, which concentrates virus, to isolate extracellular vesicles and particles from the culture medium and used in silico as a SERS substrate to measure the SERS spectra of different extracellular vesicles and used the PCA-SVM method to classify the data. The results showed that the method is capable of classifying extracellular vesicles and particles of different cancers with an accuracy of 85% and precision and sensitivity of up to 80%.(Figure 4)[68] P. Camey et al. adopted a plasmonic gold nanocluster based on a quartz microfiber matrix as a SERS substrate, which are modified with cysteamine to enhance the adsorption of anionic EVs on the surface, allowing easier and more efficient SERS signal acquisition of EVs. The authors used the PCA-QDA classification method to analyze the collected SERS signals for the rapid diagnosis of cancer. The method was tested and achieved 87% accuracy, 100% sensitivity and 70% specificity for the diagnosis of patients with head and neck cancer.[69] Yang et al. used Ag nanoparticles embedded in multilayer black phosphorus nanosheets (Ag/BP-NS) for SERS signal acquisition of different tumor exosomes, then classified them by SVM method. The classifier can not only distinguish between different tumor exosomes, but also effectively avoid the effect of PBS on signal acquisition.[70] Hisey et al. used a SERS-active thin film as a substrate to detect exosome signals, and then used CNN to classify the obtained SERS signals. This novel detection method may be one of the methods of liquid biopsy for preeclampsia.[71] Venkatakrishnan and colleagues used a dual machine learning approach of PCA-ANN to classify the SERS signal of NK cell vesicles in glioblastoma, by which a diagnosis of glioblastoma can be made with only 5 uL of peripheral blood.[72]

3.2. The application in identifying diseases

SERS combined with ML can be applied to the detection and analysis of proteins in body fluids such as urine, saliva and blood, and can be further applied to the identification of proteins with other biomolecules, which has promising clinical applications.[73]

3.2.1. Identification of the diseased and healthy population

Blood tests, tissue biopsies and imaging tests are routinely used in hospitals to diagnose disease, but these tests are often time-consuming, expensive and highly demanding on the testers, which means that new tests need to be developed.[74–76]

Biomarker is a species of protein. From the perspective of identifying a disease, a biomarker for a particular disease is likely to be a virus, an inflammatory factor or a specific amino acid. Biomarkers can often be used in the identification and diagnosis of diseases or as specific targets of drug therapy, which are closely related to the development of precision medicine.[77] As a rapid, non-invasive diagnostic technique, SERS has improved its diagnostic accuracy when combined with machine learning, which enables the technique to be used in classifying. The spectral differences between diseased and normal samples are not enormous when SERS is used as a label-free method. Machine learning, a data analysis method, can extract small differences in order to distinguish between the diseased and the normal.[28] Researchers can find the chemical origin of the spectral signature peaks observed in body fluid samples such as serum and further explore whether inter-individual variation in the substance of that origin covers the spectral differences in the subject group to confirm the biomarkers.[78]

Routine screening procedures for colorectal cancer are time-consuming and uncomfortable colonoscopy and faecal immunochemical tests. Gong et al. found that the concentrations of tyrosine and phenylalanine in serum samples were a major difference between colorectal cancer patients and healthy individuals and that this difference could be well demonstrated in the SERS spectra. The authors analyzed the spectral data obtained using the PCA method and the SVM method respectively, showing that the SVM method had better classification results when using serum samples to identify CRC.[79] Early lung cancer is screened by low-dose CT with radiation risks, a method that is not friendly to special groups such as pregnant women. Nevertheless, Yin et al. employed the SVM method to analyze the SERS spectra of serum samples and showed good screening for lung adenocarcinoma nodules with an accuracy of 93.33%. This is not only highly accurate, which assists in early screening, but also has the advantage of being non-invasive and causing no harm to the patient.[80] Furthermore, both the PCA-RF algorithm and the PCA-kNN algorithm developed by Elec et al. have good classification results for serum samples of kidney cancer.[81]

Apart from blood samples, the method applies to the analysis of a variety of other body fluid samples such as tears, urine, saliva, etc. Omar and colleagues applied PCA-SVM to the data analysis of the SERS profiles of saliva samples, which could be classified by the presence or absence of NS1 spectral peaks since this flavivirus biomarker was also detectable in saliva.[82]

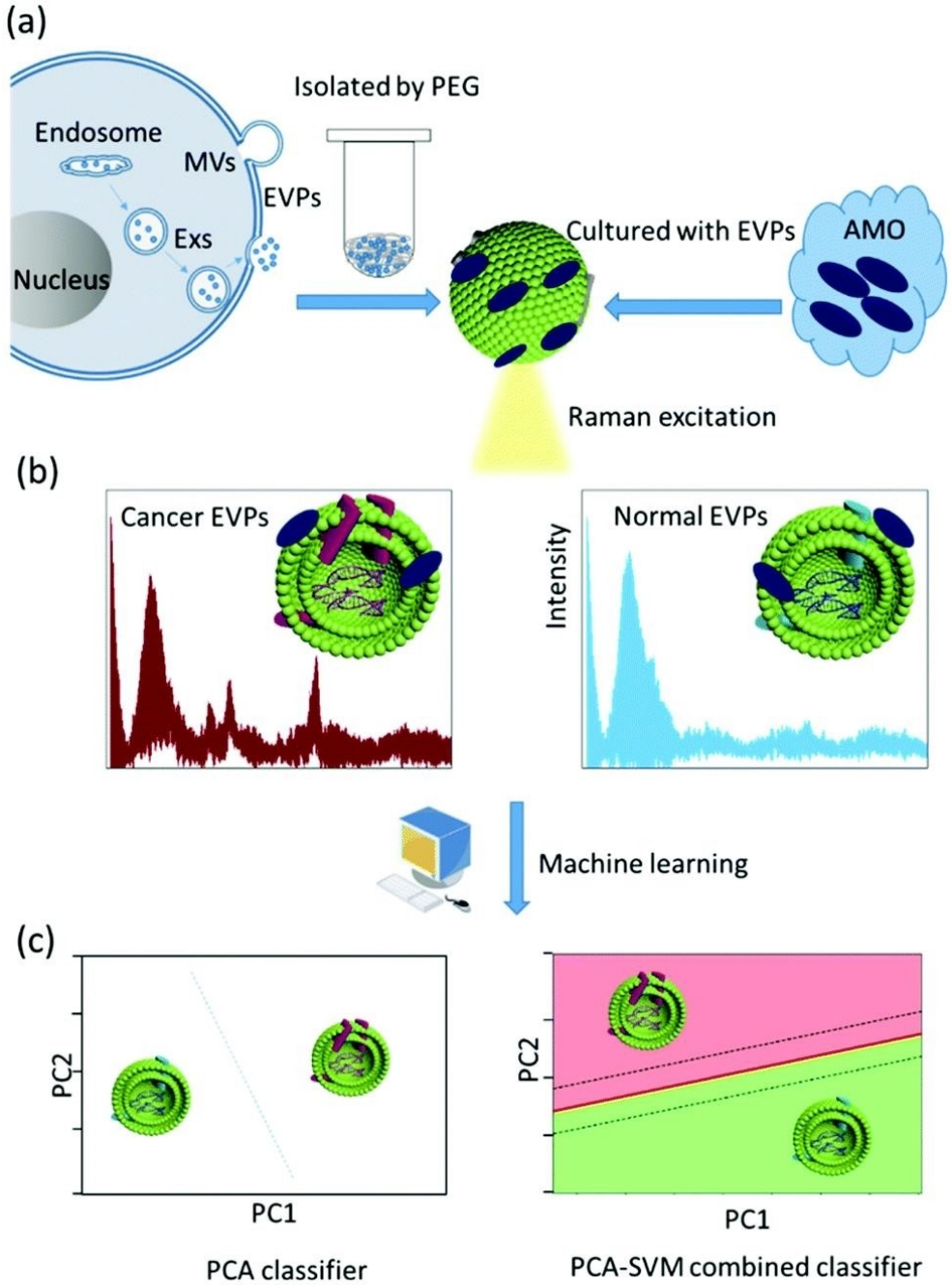

Figure 4. Schematic of the isolation, detection, and classification of EVPs. (a) EVPs were released by cells, isolated with PEG, and cultured with AMO for Raman enhancement. The "MVs" and "Exs" refer to "micro- vesicles" and "exosomes", respectively. (b) The SERS spectra of cancer and normal EVPs. (c) The classification results of EVPs by PCA and PCA-SVM classifier. Reproduced from Ref. [68] Copyright (2022), with permission from Royal Society of Chemistry.

Looi et al. applied Screen-CNN to capture the characteristic peaks of NS1 molecular fingerprint, and the final result achieved 100% performance of the classifier model for all performance metrics compared to conventional ELISA kits.[83] Liu et al. used dynamic surface-enhanced Raman spectroscopy (D-SERS) for the trace detection of drug residues in urine and SVM for data analysis of the obtained spectral information, which was used to confirm the detection of the presence of the target drug in urine.[84]

In clinical diagnosis, the importance of diagnostic accuracy is one of the most important indicators to consider, and the PLS method is frequently considered as an algorithm to minimize the sum of error squares by finding some absolutely unknowable truth values in a minimalist way. Lin and his team used PCA and PLS methods to extract SERS spectral features based on SERS spectra of liver cancer (LS), nasopharyngeal cancer (NC) and normal volunteer serum proteins purified by membrane electrophoresis, and then used SVM for further diagnostic classification. Lin's final experimental results showed that the

PLS-SVM method has excellent classification and prediction ability between different diseases, achieving an accuracy of 95.09% in the experiments (Figure 5).[85]

LDA as a clustering method is also suitable for application in the classification of clinical diagnoses. Neagoe et al. used PCA-LDA to extract features and classify patients with breast cancer, colorectal cancer, lung cancer, ovarian cancer and oral cancer. The final results showed that the accuracy rate of oral cancer diagnosis is 88%, colorectal cancer with 86% accuracy, ovarian cancer with 80% accuracy, breast cancer with 76% accuracy and lung cancer with 59% accuracy. Although there is still some way to go before it can be used in the clinical setting, it shows good promise.[86] Hepatocellular carcinoma (HCC) is already the only cancer that can be diagnosed by imaging techniques without histological formalities, but diagnostic imaging techniques have limitations in terms of sensitivity, cost and patient compliance. Bonifacio's subject group combined the RDCV method with the PCA-LDA method to analyze SERS

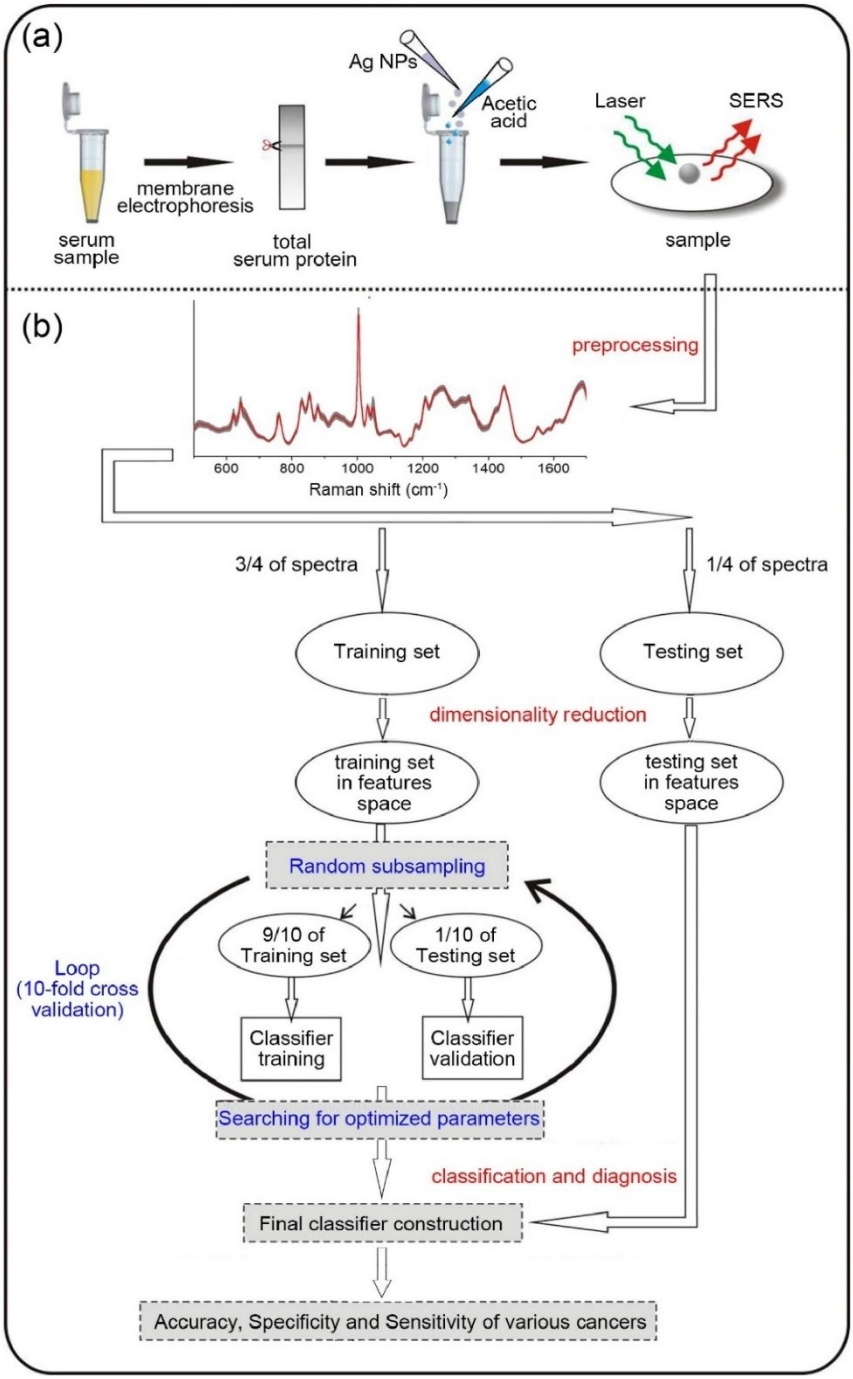

Figure 5. (a) Sample preparation and SERS measurement. (b) Schematic overview of the procedure for spectra classification and diagnosis. Reproduced from Ref. [85] Copyright (2022), with permission from The Optical Society.

spectra on serum samples from patients with hepatocellular carcinoma and healthy individuals, and this method achieved an average accuracy of 81 %.[87]

In addition to using conventional SERS spectra for machine learning analysis, first-order derivative transformation of SERS spectra can also be a means of improving detection performance in specific cases. The area under the ROC curve for the PCA-LDA was 0.927, compared to 0.860 without first-order derivatization, which was a significant improvement in the accuracy of the discrimination.[88]

When choosing a machine learning algorithm, it is not necessary to choose the most popular algorithm to operate on the model. In classifying healthy people as well as people with bladder cancer, Elec et al. found that the naïve Bayes demonstrated better performance than logistic regression and random forest models when dealing with a small number of samples.[89]

3.2.2. Identification of different types or periods of the same disease by label-free method

Biomarkers can also identify different stages of the same disease, making up for the defect of blood detection due to the lack of some important biomolecules in some stages, such as circulating tumors only exist in the stage of metastasis. Different periods of disease progression can lead to different biomarkers, which in turn can lead to changes in the SERS spectra. K. Maiti and his team, who used AuNPs as a SERS substrate to obtain SERS spectra and used SVM to classify the resulting spectra, successfully completed the classification of cervical exfoliated cells from normal, high-grade intraepithelial lesions and cervical squamous cell carcinoma in three samples of cervical cancer, including single cells, cell pellets and extracted DNA. In particular, the method of classifying the SERS spectra of extracted DNA using SVM achieved an accuracy of 92% (Figure 6).[90]

Different types of the same disease can also be identified using similar methods. Choi et al. applied the SILAR technique for deposition of golden nanoparticles on paper strips and such a stable substrate to detect SERS in human tears, and then used PCA-SVM, a machine learning tool, to classify the resulting SERS profiles to identify adenoviral conjunctivitis, herpes simplex keratitis and herpes zoster keratitis and healthy human eyes.[91]

3.2.3. Developing new immunoassay techniques

Traditional immunoassay methods rely on highly specific molecular recognition between antigens and antibodies but have the disadvantages of being complex and costly to operate. The combination of SERS and machine learning can compensate for the shortcomings of traditional immunoassay methods and is a rapid, trace and non-invasive means of analysis.[92] Wang's team fabricated a sandwich immunoassay by using AgNPs as immune probes and SiC@Ag@AgNPs as SERS substrate can realize the simultaneous monitoring of PSA. PSMA and hK2 biomarkers in prostate cancer. Combined with a linear SVM pattern recognition model, this immunoassay

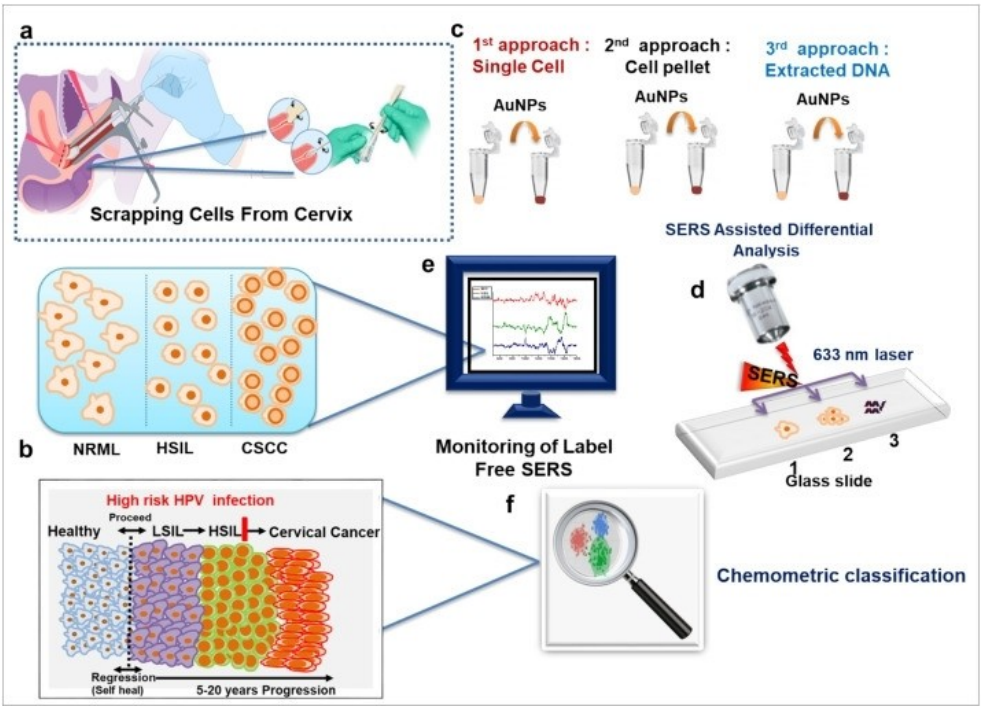

Figure 6. Schematic illustration of experimental design for differentiating three grades viz. normal (NRML), high grade intraepithelial lesion (HSIL), cervical squamous cell carcinoma (CSCC) using SERS., a) Scrapping cells from the cervix using cytobrush, b) progression pattern of cervical cancer c) Set 1: single cell, Set 2: cell pellet, Set 3: extracted DNA (mixed with AuNPs), d) independent SERS analysis of 1) single cell, 2) cell pellet, 3) extracted DNA in glass slide, d) empirical signal monitoring of the three grades f) chemometric analysis. Reproduced from Ref. [90] Copyright (2020), with permission from Elsevier.

method can detect the very low detection limit of these three biomarkers, showing the excellent performance of this method in clinical application.[93]

3.3. Study of the biochemical action of specific substances

In the conventional approach, the spectral expression of some cellular components is not strong enough to be used for reliable classification. However, combining machine learning with SERS allows for the identification of previously weak feature signals, enabling the identification of specific biochemical responses in cell. Kneipp and team used SERS to identify ingredients of lipid aggregates in living fibroblasts, measuring the spectra of three tricyclic antidepressants (TCA) before and after induction of the cell, respectively, and perform random forest (RF) analysis, concluded that the majority of sphingolipids accumulate in lysosomes following drug induction and also yielded information on other accumulated lipids.[94]

3.4. Enhancing SERS capabilities in semi-quantitative measurements

Due to lack of stability, SERS as a qualitative measurement tool has certain disadvantages in terms of quantification. As a result of the efforts of researchers, SERS is gradually expected to become a trusted tool for semi-quantitative measurement and analysis.[95,96] The use of machine learning for SERS contributes to the application of SERS in the field of semi-quantitative measurements. Barman et al. used Ag/SiNWs as Raman substrates to measure the amount of glycated albumin (GA) in blood and used partial least squares (PLS) and regression with leave-on sample cross-validation (LOOCV) to make predictions about glycated hemoglobin concentrations, reinforcing the quantitative capability of SERS as a qualitative tool.[97] Masson et al. used SERS to monitor seven metabolites around Hela and HUVEC cells, and 1D CNN was used to analyze the data from the SERS spectra. The results show that the method can show to some extent the gradients of multiple metabolites in the extracellular environment and can be able to provide detailed information on metabolite uptake and secretion.[98]

4. Conclusion and Outlooks

In this review, we focussed on the application of SERS combined with ML in medicine, including recognition of biological molecules, rapid diagnosis of diseases, developing new immunoassay techniques, and enhancing SERS capabilities in semi-quantitative measurements. We identified that the analysis of SERS is more accurate when combined with ML algorithms, which is also increasing the breadth and depth of SERS applications in the medical field.

We discovered that the application of machine learning combined with SERS to medical problems in previous studies not only overcomes the shortcomings of traditional clinical testing tools, which are time-consuming, expensive and demanding for the testers, but also provides highly accurate results in classifying the presence or absence of disease and the type of disease. The method has demonstrated superior performance in comparison with other traditional methods.

At present, as a mature dimensionality reduction method, PCA is widely used in SERS data processing. Combining PCA with other machine learning algorithms has become the main consideration for spectral data processing by many researchers, and a large number of related studies and articles have emerged this year.

Despite numerous merits, persistent developments are still needed. For illustration, the PCA and SVM mentioned in the paper apply to different sizes and dimensions of data sets, and we need to analyze more experiments to find out the algorithms that can be more universally applied to SERS analysis, to obtain the large-scale application of this method in medical analysis.

In the method of combining machine learning with SERS, this approach suffers from drawbacks such as uncertainty, probabilistic and wireless datasets of the algorithm due to the insufficient amount of data in the training set, poor quality and overfitting. These challenges of machine learning still need to be overcome by a wide range of researchers through continuous research and experimentation, so that the combined machine learning and SERS approach can be better applied in clinical settings.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Keywords: chemometrics · machine learning · medicine · Raman spectroscopy · statistical spectral analysis

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