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INVENT

SCOPE OF LABEL-FREE RAMAN IN EARLY DISEASE DIAGNOSIS

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INTRODUCTION

Raman spectroscopy is a technique used to study the vibrational modes of molecules, and is a non-destructive method of chemical analysis especially for functional fingerprint identification. It is based on the Raman effect, which was discovered by the Indian physicist Sir C.V. Raman in 1928. The Raman effect arises from the interaction between the electromagnetic radiation and the polarizability of the sample molecules. When the incident light interacts with a molecule, it can cause the molecule to vibrate or rotate. If the frequency of the incident light is close to the frequency of the vibrational or rotational mode of the molecule, then the molecule can absorb energy from the incident light and shift to a higher energy state. This results in the inelastic scattering of the light, which is known as Raman scattering. The Raman scattering spectrum contains peaks at frequencies corresponding to the vibrational and rotational modes of the molecules in the sample. These peaks are called Raman peaks, and they provide information about the chemical composition and molecular structure of the sample. Raman spectroscopy is a powerful technique for analyzing a wide range of materials, including solids, liquids, and gases. It is widely used in chemistry, physics, biology, and materials science, among other fields. For generating Raman shift or so called Raman spectrum, a laser is used to irradiate a sample and the scattered light is analyzed for frequency shifts using a spectrometer. The resulting spectrum provides information about the vibrational modes of the molecules in the sample, which can be used to identify the chemical composition and molecular structure of the sample. This technique has many applications, including in the analysis of pharmaceuticals, polymers, minerals, and biological samples. It is also used in forensics, art conservation, and the study of materials under extreme conditions such as high pressure or temperature.

One of the major advantages of Raman spectroscopy is that it is a label-free technique, [1] meaning that it does not require the use of external markers or labels to study samples. This is in contrast to other techniques such as fluorescence spectroscopy, which require the use of fluorescent labels to visualize and analyze samples. The label-free nature of Raman spectroscopy makes it a versatile tool for studying a wide range of chemical and biological samples, including cells, tissues, and biological fluids. It can be used to analyze the composition, structure, and interactions of molecules and biomolecules, such as proteins, lipids, nucleic acids, and carbohydrates.[2] In addition to its label-free capabilities, Raman spectroscopy offers several other advantages, including high sensitivity, high spatial resolution, and the ability to analyze samples in situ, without the need for sample preparation or destruction. It is also a non-destructive technique, meaning that samples can be analyzed multiple times without damage. Label-free Raman spectroscopy has many applications in both basic and applied research, including in the analysis of biological tissues and cells, the characterization of polymers and materials, and the detection of environmental contaminants. Its ability to analyze samples without the need for external labels or markers makes it a powerful tool for a wide range of research fields (figure1)

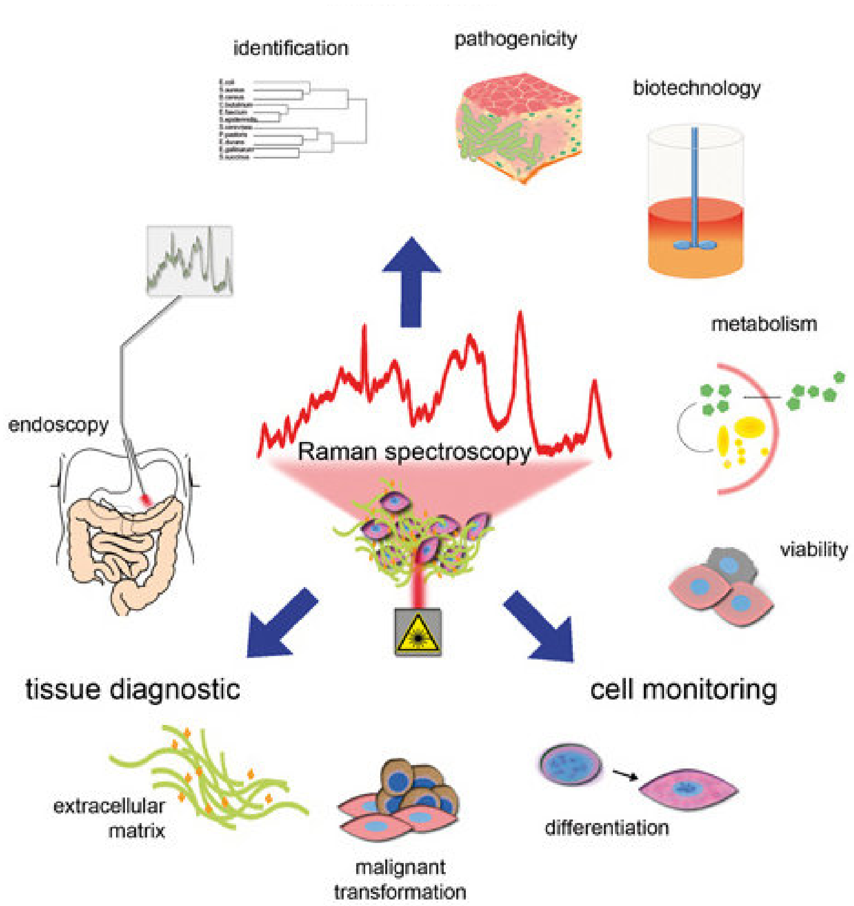

Figure 1: Schematic representation of various applications of Raman spectroscopy figure adapted from wiely.[3]

EARLY DISEASE DIAGNOSIS

Label-free Raman spectroscopy is a powerful tool that has the potential to revolutionize early disease diagnosis. Raman spectroscopy is an analytical technique that can offer insights into the overall chemical composition and functional group fingerprints of the structure of a sample, without causing any damage to it. In label-free Raman spectroscopy, the Raman fingerprints were obtained without the use of labeling agents, and the sample is analyzed in its natural state. This technique has been applied successfully to diagnose various diseases in their early stages of communicable and non-communicable diseases, including cancer, Alzheimer's disease, diabetes etc. This technique has many advantages over traditional fluorescence labeling techniques, including being non-invasive, non-destructive, and not requiring any sample preparation. It can detect changes in the biochemical composition of cells or tissues associated with disease development before physical symptoms become apparent. The ability to detect these diseases early is critical for effective treatment and better patient outcomes.

a. Cancer

In cancer diagnosis, label-free Raman spectroscopy has been used to detect the molecular changes associated with the development of cancer cells. This technique can detect changes in the biochemical composition of cells, such as changes in protein expression, lipid content, and DNA structure. These changes can be detected before any physical symptoms of cancer are present, enabling early detection and treatment. This is because cancer cells have different biochemical properties than healthy cells, which can be detected by Raman spectroscopy. [4]However, Raman spectroscopy still faces some challenges in cancer diagnosis, such as low sensitivity and specificity, and the need for further validation studies to confirm its accuracy and reliability. Nonetheless, it is a promising technique for non-invasive cancer diagnosis and has the potential to be used in combination with other imaging techniques to improve cancer detection and diagnosis. To diagnose various tumors, Raman spectroscopy data is collected from tumor tissues, model cell lines, biological fluids and biomarkers, and processed using statistical, machine learning, and the advanced deep learning methods. Raman spectroscopy has shown great promise as a non-invasive, label-free diagnostic tool for various types of cancers.[5] It works by analyzing the molecular composition of tissues and cells based on their unique vibrational spectra. In the case of bladder cancer, Raman fingerprints have been used to detect and differentiate between malignant and benign bladder tissue. Similarly, it has been used to diagnose breast cancer by identifying the differences in the molecular composition of cancerous and non-cancerous breast tissue. It has also been employed to detect brain tumors and differentiate between different types of brain cancer, such as glioblastoma and meningioma. Similarly in cervical cancer, Raman signatures have been used to identify the molecular markers associated with cancerous cells, which can help in early detection and diagnosis. Furthermore, Functional fingerprints from Raman spectra have been shown to be effective in diagnosing colorectal cancer by analyzing the molecular composition of tissue samples. In addition, it has been used to detect and differentiate between malignant and benign esophageal tissue and to diagnose gastrointestinal cancers. Liver cancer is another type of cancer that has been diagnosed using Raman spectroscopy. The technique has been used to analyze liver tissue samples and identify the presence of cancerous cells. It has also been used to diagnose lung cancer by analyzing the molecular composition of exhaled breath condensate. Similarly, it has been used to diagnose oral cancer by analyzing tissue samples obtained from the oral cavity. In addition to the above-mentioned cancers, Raman spectroscopy has been used to diagnose nasopharyngeal carcinoma, laryngeal carcinoma, and ovarian cancer. Overall, Raman spectroscopy has shown great potential as a diagnostic tool for various types of cancer, and ongoing research is exploring its further applications in cancer diagnosis and treatment.[6]

b. Alzheimer's Disease

In Alzheimer's disease, label-free Raman spectroscopy has been used to identify changes in brain tissue associated with the disease. This technique can detect changes in the structural fingerprints and conformational changes of brain tissue, such as the presence of amyloid plaques and tau protein tangles, which are characteristic of Alzheimer's disease. Dementia impacts the memory, cognitive functions, and behavior of elderly individuals, with Alzheimer's disease being the most prevalent type and accounting for approximately 65% of dementia cases. The accumulation of amyloid-beta, also known as amyloid plaque, and hyperphosphorylated tau deposits are the primary pathological indicators of Alzheimer's disease. Current diagnostic methods involve targeting amyloid-beta in the brain, cerebrospinal fluid, or blood, but these techniques only provide indirect information about the pathological substrate. The most conclusive diagnosis of Alzheimer's disease is obtained through (immuno-) histochemical staining on brain tissue, but this method is time-consuming, invasive, and risky. Raman spectroscopy has the potential to provide valuable diagnostic options for Alzheimer's disease, as it can be used to detect changes in the molecular composition and structure of biological samples. In particular, Raman spectroscopy has been used to detect the accumulation of beta-amyloid proteins, which are believed to play a role in the development and progression of Alzheimer's disease. Beta-amyloid proteins are known to form aggregates that can lead to the formation of plaques in the brain, which are a hallmark of Alzheimer's disease. This technique can also be used to detect changes in lipid composition and metabolism that may be associated with Alzheimer's disease. For example, Raman spectroscopy has been used to detect changes in the ratio of saturated to unsaturated fatty acids in brain tissue from Alzheimer's disease patients, which may be related to changes in membrane fluidity and neuronal function. These techniques could offer new insights into the composition of the pathological substrate and potentially enable the detection and monitoring of pathology. In general, Raman spectroscopy is a promising label-free technique that has the potential to provide valuable diagnostic options for Alzheimer's disease. Further research is needed to fully understand the diagnostic potential of Raman spectroscopy, as well as to develop robust and reliable methods for its use in clinical settings.

c. Diabetes

In diabetes diagnosis, label-free Raman spectroscopy has been used to detect changes in the composition of blood and urine samples. This technique can detect changes in glucose and lipid levels, which are indicators of diabetes. When monitoring glucose noninvasively, the Raman spectrum captured provides insight into variations in lipids content, protein conformational changes, nucleic acids interaction, disparities in carbohydrate content, including glucose. Despite glucose being a weak Raman active molecule, meaning it has a weak power of generating Raman signals compared to its concentration in tissue. To solve this, advanced data analysis software is clubbed with Raman techniques to enable more accurate detection. Diabetes is a condition characterized by high blood glucose levels, but there are currently no other disease predictive signature molecules for this disease. Currently biomarkers such as hemoglobin A1c variations and fasting blood glucose level are only used when symptoms of diabetes are observed. Birech et al.[7] demonstrated the use of Raman spectroscopy for quick screening of diabetes prediction (both diseased and possibility) in human or rat blood samples. Raman spectrum variations were observed for glucose and variations in fingerprints of leucine and isoleucine amino acids in blood, where variations in the intensities of assigned bands serve as references. The research proposes that Raman spectroscopic patterns of the selected biomarkers could be used as an alternative approach to compare the effectiveness of known and novel anti-diabetic drugs. An intriguing aspect is the application of Raman spectroscopy in screening blood samples for changes in the levels of leucine and isoleucine amino acids as an indicator of pre-diabetes. This could facilitate timely interventions to prevent the onset of diabetes.

CONCLUSION

Raman spectroscopy holds promise in elucidating the molecular mechanisms underlying diseases and providing unbiased, measurable molecular information for diagnosis and treatment evaluation. Several studies have demonstrated the ability of Raman spectroscopy to characterize tissues. However, for it to be clinically relevant, it is crucial to develop extensive spectral databases and tissue classification methodologies that can be compared to current diagnostic standards. Establishing best practices for data acquisition, processing, and classification is essential. Fluorescence, which can compete with Raman scattering, can affect the interpretation of Raman spectra. Therefore, preprocessing the raw data can eliminate unwanted signals, enhance Raman spectral features, and generate more consistent data for qualitative and quantitative analysis. The effectiveness of tissue classification results in Raman spectroscopy is highly dependent on the choice of preprocessing strategy. Thus, it is essential to develop the most appropriate preprocessing techniques. Furthermore, developing classification algorithms for diagnostic assessment requires careful consideration, and validation studies are necessary to establish their applicability to in vivo tissues. Machine learning algorithms, especially deep learning, hold significant potential in automating cancer diagnosis and identification, detecting molecular patterns among cancer types, aiding in margin detection, and predicting cancer aggressiveness. In addition to algorithm development, research on the laser-tissue interaction that can lead to tissue damage is necessary to ensure the safe and effective use of Raman spectroscopy in clinical applications. While increasing the light intensity can lead to more Raman signals, it is important to note that there is a threshold beyond which tissue damage can occur. To make Raman spectroscopy a robust and reliable tool for clinical applications, it is necessary to develop comprehensive spectral databases, effective tissue classification methodologies, and instrumentation with improved resolution, shorter collection times, and higher accuracy. These developments will enhance the sensitivity and specificity of Raman spectroscopy in diagnosing diseases and enable its wider use in clinical settings.

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