

# Review on the Application Progress of Spectroscopic Techniques in Periodontal Diseases

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## ABSTRACT

Periodontal disease is a common oral disorder that affects the tissues surrounding teeth, including the gums, periodontal ligament, and alveolar bone. Traditional diagnostic methods such as clinical examinations, X-ray imaging, and periodontal probing have limitations in early diagnosis and disease monitoring. In recent years, spectroscopic techniques, as non-invasive and highly sensitive analytical tools, have gradually demonstrated great potential in the diagnosis and treatment of periodontal diseases. This article reviews the application progress of spectroscopic techniques in the field of periodontal diseases, with a focus on the principles, application examples, and the latest research findings of Raman spectroscopy, fluorescence spectroscopy, near-infrared spectroscopy, and other techniques. These techniques provide detailed information about the composition and structure of tissues by measuring the interaction between light and biological tissues, which is helpful for the early detection of periodontal diseases, the evaluation of treatment effects, and the monitoring of disease progression. Through the application of these techniques, we can comprehensively understand the pathological mechanisms of periodontal diseases, develop more effective diagnostic tools, and provide more precise guidance for clinical treatment.

## 1. INTRODUCTION

Periodontal disease is a prevalent oral condition that impacts the tissues around teeth, namely the gingiva, periodontal ligament, and alveolar bone. If left untreated, it may lead to tooth loosening and even tooth loss, severely affecting oral health and the quality of life. Early diagnosis of periodontal disease mainly relies on methods such as clinical examinations, X-ray imaging, and periodontal

probing. However, these methods have certain limitations in early diagnosis and disease monitoring. In recent years, spectroscopic techniques, as non-invasive and highly sensitive analytical tools, have shown great potential in the diagnosis and treatment of periodontal diseases.

Spectroscopic techniques provide detailed information about the composition and structure of tissues by measuring the interaction between light and biological tissues. These techniques include Raman spectroscopy, fluorescence spectroscopy, near-infrared spectroscopy, etc., and each has its unique principles and application advantages. For example, Raman spectroscopy can provide detailed information on molecular vibration modes, helping to identify the structure and composition of biomolecules; fluorescence spectroscopy provides information about the chemical environment and metabolic state of biological tissues by measuring fluorescence intensity and lifetime. The high sensitivity and specificity of these techniques give them significant advantages in the early detection of periodontal diseases, the evaluation of treatment effects, and the monitoring of disease progression.

In recent years, significant progress has been made in the application of spectroscopic techniques in the field of periodontal diseases. For example, Raman spectroscopy has been used to detect biomarkers in periodontal tissues, and fluorescence spectroscopy has been used to evaluate the oxidative stress level of periodontal tissues. In addition, near-infrared spectroscopy, due to its penetration ability in biological tissues, has been used to monitor the blood flow and oxygenation status of periodontal tissues. The development of these techniques not only improves the diagnostic accuracy of periodontal diseases but also provides a scientific basis for the formulation of personalized treatment plans.

This article summarizes the application progress of spectroscopic techniques in the field of periodontal diseases, focusing on the principles, application examples, and the latest research findings of Raman spectroscopy, fluorescence spectroscopy, near-infrared spectroscopy, and other techniques. Through these techniques, we can

comprehensively understand the pathological mechanisms of periodontal diseases, develop more effective diagnostic tools, and provide more precise guidance for clinical treatment.

## 2. INTRODUCTION TO SPECTROSCOPIC TECHNIQUES

### 2.1. Raman Spectroscopy

Raman spectroscopy is a non-destructive analytical technique based on the Raman scattering phenomenon, widely used in materials science, chemical analysis, biomedicine, environmental science, and other fields[1]. When a monochromatic light with a frequency of  $\nu_0$  irradiates a sample, most of the light undergoes elastic scattering, that is, the frequency of the scattered light is the same as that of the incident light, which is called Rayleigh scattering. However, due to molecular vibration, rotation, and other energy level transitions, a small part of the light undergoes inelastic scattering, and the frequency of the scattered light is different from that of the incident light, generating Raman scattering. Raman spectroscopy causes little damage to samples, can be used for the study of aqueous solution systems, and can provide fingerprint information of molecular structures, especially suitable for analyzing molecules with symmetric structures. In the chemical field, Raman spectroscopy is used to analyze the structures of organic and inorganic compounds; in materials science, it is used to study the lattice structure and defects of materials; in biomedicine, it is used for the structural analysis of biomolecules and disease diagnosis.

In recent years, remarkable progress has been made in the application of Raman spectroscopy in the field of periodontal diseases. Surface-enhanced Raman spectroscopy (SERS) significantly enhances the Raman signal by adsorbing the molecules to be detected on the surface of metal nanostructures, improving the detection sensitivity. SERS has important applications in the detection of biomarkers for periodontal diseases, such as the

detection of low-concentration inflammatory markers and oxidative stress markers. Inflammatory markers such as interleukin (IL-1, IL-6, IL-8) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) can trigger alveolar bone resorption and periodontal tissue destruction by activating inflammatory cells, promoting osteoclast formation, and amplifying the inflammatory response. Their levels are significantly correlated with the severity of periodontitis. Among oxidative stress markers, malondialdehyde (MDA), as a product of lipid peroxidation, reflects the degree of oxidative damage to periodontal tissues. The activities of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) initially increase compensatorily and then decrease due to excessive oxidative stress. Their changes reflect the body's antioxidant defense ability and are closely related to the degree of periodontal tissue destruction. These advancements not only improve the diagnostic accuracy of periodontal diseases but also provide a scientific basis for the formulation of personalized treatment plans.

## **2.2. Fluorescence Spectroscopy**

When a substance molecule absorbs light of a specific wavelength, electrons transition from the ground state to the excited state[3]. The electrons in the excited state are unstable and will return to the ground state in a very short time, releasing energy in the form of light and generating fluorescence. Fluorescence spectroscopy measures the relationship between the fluorescence intensity emitted by a substance and the wavelength. Fluorescence detection technology has shown broad application potential in biomedicine, environmental monitoring, and other fields due to its significant advantages. Its prominent features include high sensitivity, which can detect extremely low concentrations of fluorescent substances, enabling the capture of trace biomarkers and providing the possibility for early disease diagnosis; excellent selectivity, which can accurately distinguish target substances in complex systems by using the unique fluorescence emission and excitation spectra of different fluorescent substances, effectively avoiding interference; and fast response characteristics, which support real-time monitoring

of molecular dynamic changes, being suitable for real-time tracking of reaction processes or environmental changes, and thus enabling dynamic monitoring and precise control. In biochemistry, it is used for the labeling and detection of biomolecules such as proteins and nucleic acids; in environmental monitoring, it is used to detect pollutants; in drug research and development, it is used to study the interaction between drugs and biomolecules.

In recent years, significant progress has been made in the application of fluorescence spectroscopy in the field of periodontal diseases. Time-resolved fluorescence spectroscopy can provide information on molecular dynamics and the chemical environment by measuring the fluorescence lifetime (i.e., the time for a fluorescent molecule to return from the excited state to the ground state). Time-resolved fluorescence spectroscopy has important applications in the detection of biomarkers for periodontal diseases. The high sensitivity of time-resolved fluorescence spectroscopy can accurately capture trace inflammatory factors and oxidative stress markers in periodontal tissues, enabling early diagnosis. Its strong selectivity can accurately identify target substances in complex samples, and its fast response characteristics can track the protein folding process and the interaction between drugs and cellular targets in real-time, providing key data support for the study of the mechanisms of periodontal diseases and targeted therapy.

## **2.3. Near-Infrared Spectroscopy**

When infrared light irradiates a substance, the molecules of the substance absorb infrared light of specific wavelengths, causing transitions in molecular vibration and rotation energy levels. Different chemical bonds and functional groups have different absorption frequencies in the infrared region, forming characteristic infrared absorption spectra. Infrared spectroscopy is a rapid and convenient analytical method. Sample preparation is simple, and it can be used for the analysis of solid, liquid, and gas samples. It can provide rich information about functional groups and is widely used in the structure identification of compounds. In organic chemistry, it is used to determine the

functional groups and structures of organic compounds; in the field of polymer materials, it is used to analyze the structures and compositions of polymers; in industries such as food and pharmaceuticals, it is used for quality control and authenticity identification. In the diagnosis of periodontal diseases, near-infrared spectroscopy has significant advantages. By detecting the vibration absorption spectra of hydrogen-containing groups in biomolecules, it can sensitively capture information such as abnormal protein structures, lipid metabolism disorders, and changes in nucleic acid content during periodontal lesions, providing rich molecular-level data for the early diagnosis, disease progression monitoring, and treatment effect evaluation of periodontal diseases, and showing unique advantages in the non-invasive detection and precise diagnosis and treatment of periodontal diseases.

In recent years, significant progress has been made in the application of near-infrared spectroscopy in the field of periodontal diseases. For example, near-infrared spectroscopic imaging technology combines spectroscopic measurement and imaging technology to achieve high-resolution imaging of periodontal tissues. Near-infrared spectroscopic imaging has important applications in the detection of biomarkers for periodontal diseases, such as monitoring the blood flow and oxygenation status of periodontal tissues.

#### **2.4. Hyperspectral Technology**

Hyperspectral imaging technology adds a spectral dimension to traditional two-dimensional imaging, combining imaging technology with spectroscopic technology. It collects the reflection, emission, or transmission information of an object in continuous spectral bands, forming a three-dimensional data cube that contains spatial information and spectral information. It has extremely high spectral resolution, can obtain a large number of continuous spectral band information, and can precisely reflect the spectral characteristics of the object. At the same time, it provides rich spatial information, enabling the positioning and identification of the target. In the medical field, the technical principle of hyperspectral imaging is used for tissue imaging

and disease diagnosis. In the detection of periodontal diseases, it can capture the microstructural changes of gingival tissues through high-resolution imaging, analyze the differences in tissue texture and blood supply caused by inflammation. Its spectral analysis ability can identify the changes in the spectral characteristics of biomolecules such as proteins and lipids during periodontal lesions, thus assisting in early diagnosis and disease assessment. For example, similar to the spectral index analysis method for tracking the health status of crops in vegetation monitoring, it can be used to quantify the degree of abnormal metabolism in periodontal tissues, opening up a new path for non-invasive and precise detection of periodontal diseases.

### **3. APPLICATION OF SPECTROSCOPIC TECHNIQUES IN THE DIAGNOSIS OF PERIODONTAL DISEASE**

#### **3.1. Application of Raman Spectroscopy in the Diagnosis of Periodontal Diseases**

Raman spectroscopy has significant advantages in the diagnosis of periodontal diseases. Zhang J used a portable Raman spectrometer to collect Raman spectral data of subgingival plaques from 20 patients with chronic periodontitis complicated by type 2 diabetes, 23 patients with simple chronic periodontitis, and 23 healthy adults[2]. Eight common machine learning algorithms were used to construct classification models to compare and identify the spectral characteristics of the three types of subgingival plaques. The results showed that the linear discriminant analysis model performed best in distinguishing the three types of subgingival plaques. The classification system constructed by combining this technology with machine learning can effectively identify the differences in subgingival plaques between patients with chronic periodontitis with or without type 2 diabetes and healthy individuals, and is expected to be integrated into clinical practice as a screening or diagnostic tool in the future. Raman spectroscopy

can accurately detect the dynamic changes of molecules such as proteins, lipids, and nucleic acids by capturing the vibration signals of biomolecules in periodontal tissues. In the evaluation of treatment effects, by comparing the Raman spectral data before and after treatment, it can visually show the changes in inflammation-related molecules in periodontal tissues. For example, the reduction of collagen degradation products after treatment directly reflects the tissue repair and regeneration process, providing objective molecular-level evidence for clinical efficacy judgment. The research of Zhang J and other scholars further verified the effectiveness of the above method. By obtaining the spectral images of subgingival plaques of different populations with a portable Raman spectrometer and based on the differences in the characteristic signals of biomarkers (such as collagen degradation products), the constructed classification model not only achieved accurate differentiation of the three types of samples but also demonstrated the application potential of evaluating treatment responses through spectral comparison before and after treatment. Given its high sensitivity and specificity, Raman spectroscopy has significant clinical transformation value in the early diagnosis, disease monitoring, and efficacy evaluation of periodontal diseases, showing broad application prospects.

### **3.2. Application of Fluorescence Spectroscopy in the Diagnosis of Periodontal Diseases**

Fluorescence spectroscopy has significant advantages in the diagnosis of periodontal diseases. The pathological process of periodontal diseases involves changes in multiple biomolecules such as proteins, lipids, and nucleic acids. Fluorescence spectroscopy can provide detailed electron transition information of these molecules, helping to identify and quantify biomarkers in periodontal tissues. Yan Y J used a hyperspectral imaging microscope to obtain the fluorescence spectral data of *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, and *Streptococcus mutans*. In response to the three key problems found during the experiment - the stray light interference introduced by the mercury line light source, the red fluorescence background generated by the

borosilicate glass slide, and the photobleaching phenomenon of the fluorescence signal of *Porphyromonas gingivalis*, targeted solutions were proposed[4]. A bacterial classification method based on the ratio of specific spectral intensities (510/635nm and 500/635nm) under 405nm excitation light was constructed. The classification sensitivity and specificity of this method for the three bacteria reached approximately 99%. The results show that fluorescence spectroscopy can be used as a rapid and efficient means of oral health monitoring. By analyzing the dynamic changes of fluorescence signals, it can achieve early diagnosis and disease course tracking of periodontal diseases. In addition, this technology has application potential in the evaluation of periodontal treatment effects. By comparing the fluorescence spectral characteristics before and after treatment, it can quantify the changes of inflammatory markers in periodontal tissues, providing an objective basis for clinical efficacy judgment. Given its outstanding sensitivity and specificity, fluorescence spectroscopy shows broad clinical application prospects in the field of periodontal disease diagnosis and treatment.

Nie M et al. explored the research progress of photodynamic therapy (PDT) in the antibacterial effect on periodontal pathogens and its impact on multiple aspects of periodontal health, including periodontal immune cells, human gingival fibroblasts, gingival collagen, inflammatory mediators, periodontal cytokines, vascular oxidative stress, vascular behavior, and alveolar bone health[6].

Ushakou D et al. used fluorescence decay curve analysis technology to accurately measure the total decay rate constant of excited molecules. This method has potential application value in the diagnosis of periodontal diseases. By analyzing the decay kinetic characteristics of fluorescent substances in diseased tissues, it is expected to achieve early detection of changes in the periodontal tissue microenvironment, providing new quantitative indicators for non-invasive diagnosis and disease assessment. The constructed



model has been verified in numerical simulation cases, and the research further discussed the experimental data in combination with this model[7].

Li C et al. pointed out in their research in the Journal of the American Chemical Society that this technology, with its characteristics of high tissue penetration depth and low background fluorescence interference, can achieve high-resolution visualization of the biological tissue microenvironment. The newly developed fluorescent probe and imaging system can accurately capture the fluorescence signal changes of metabolic products or inflammatory markers in periodontal tissues. By analyzing parameters such as the fluorescence intensity and decay kinetics in the near-infrared II region, it is expected to identify periodontal tissue lesions at an early stage, providing a new strategy for minimally invasive and real-time diagnosis and efficacy evaluation of periodontal diseases and helping to break through the limitations of traditional detection methods[8].

Gatin E's research proposed that during the intraoperative in vivo measurement of specific patients, the target peaks of Raman spectra for calcium phosphate and other phosphate reference compounds were located[9]. It was found that there is a correlation between the fluorescence characteristics of bone samples and the collagen content. This feature provides an important basis for the multi-dimensional assessment of bone quality and further reveals the core role of the collagen matrix as a carrier for calcium phosphate deposition in the complex bone mineralization process. The high sensitivity and specificity of fluorescence spectroscopy enable it to provide detailed molecular information, helping to develop personalized treatment plans. For example, by analyzing specific biomarkers in the fluorescence spectrum, it is possible to determine the patient's response to specific treatments and adjust the treatment plan to achieve the best results.

### **3.3. Application of Near-Infrared Spectroscopy in the Diagnosis of Periodontal Diseases**

Lee U H et al. developed a novel

donor-acceptor-donor (A - D - A) type non-fullerene acceptor (NFA) with near-infrared light responsiveness, which has greatly promoted the development of organic photovoltaics (OPV) and has great application potential for sensitive near-infrared organic photodetectors (OPD)[5]. The research also looked ahead to the development direction of periodontitis detection in the future. Near-infrared spectroscopy provides detailed information about disease progression and treatment effects by detecting the blood flow and oxygenation status in periodontal tissues. Near-infrared spectroscopy can provide detailed absorption information of these molecules, helping to identify and quantify biomarkers in periodontal tissues. For example, near-infrared spectroscopy can detect the absorption changes of hemoglobin and collagen in periodontal tissues, and these changes occur in the early stages of periodontal diseases. By analyzing the changes in these absorption signals, early diagnosis and disease monitoring of periodontal diseases can be achieved. In addition, near-infrared spectroscopy can also be used to evaluate the effects of periodontal treatment. For example, by comparing the near-infrared spectra before and after treatment, changes in the blood flow and oxygenation status of periodontal tissues can be observed, thereby evaluating the treatment efficacy. Near-infrared spectroscopy, with its high sensitivity, can capture the subtle changes of inflammatory markers and oxidative stress products in saliva at the early stage of periodontitis, achieving early warning. By using specific spectral fingerprints, it can accurately distinguish the biomolecular characteristics of healthy and diseased tissues, avoiding misdiagnosis. Its characteristics of no need for complex sample processing and fast detection provide an efficient solution for large-scale screening and dynamic monitoring of periodontal diseases.

## **4. APPLICATIONS OF SPECTROSCOPIC TECHNIQUES IN THE RESEARCH OF PERIODONTAL DISEASES**

#### **4.1. Application of Raman Spectroscopy in the Research of Periodontal Diseases**

Raman spectroscopy provides detailed information on molecular vibration modes by measuring the Raman scattering that occurs when photons interact with biological tissues. The research of Timchenko E focused on exploring the use of Raman spectroscopy to study the changes in tooth tissues affected by periodontitis, analyzing the alterations in tissue components when teeth have periodontitis, and investigating the application value of Raman spectroscopy in the diagnosis and research of periodontitis[10]. It was discovered that in teeth with periodontitis, the proline and hydroxyproline in the cementum and root dentin underwent changes, which were manifested through the characteristic peaks of the Raman spectrum. This indicates that Raman spectroscopy can detect the chemical composition changes in tooth tissues caused by periodontitis. Raman spectroscopy can serve as an effective tool for studying the impact of periodontitis on tooth tissues, providing a new method and basis for the diagnosis and disease assessment of periodontitis, and contributing to a deeper understanding of the pathological mechanisms of periodontitis.

#### **4.2. Application of Fluorescence Spectroscopy in the Research of Periodontal Diseases**

Fluorescence spectroscopy provides information about molecular structures and chemical compositions by measuring the wavelengths and intensities of the excitation and emission light[11]. Scholars such as Joseph B conducted a systematic review of relevant studies in databases including Medline, Embase, Cochrane Library, Web of Science, Google Scholar, and Scopus from January 2000 to June 2021. In the research of periodontal diseases, information on molecular structures and chemical compositions is of great significance. Most studies have shown that autofluorescence spectroscopy can detect multiple diseases based on fluorescence, including early dental plaque. As a non-invasive technique, it is a feasible and patient-friendly clinical tool for the early detection of dental biofilm plaques, and its precise removal directly contributes to the prevention of periodontal diseases. This is of great importance for

understanding the causes of periodontal diseases and selecting appropriate antibacterial treatment plans. Fluorescence spectroscopy can also monitor the dynamic changes in the activity of biofilms in real time. For example, by detecting the intensity changes of fluorescent substances in biofilms, the metabolic activity and growth status of biofilms can be evaluated, providing a basis for the early diagnosis and treatment of periodontal diseases. Based on the fluorescence properties of substances, fluorescence spectroscopy has advantages such as high sensitivity and non-destructive detection. This technique can identify the distribution of components such as bacteria and polysaccharides in periodontal biofilms through fluorescence probe labeling. It can also monitor the dynamic changes of fluorescent substances during metabolism, explore the metabolic activity of microorganisms in biofilms, and provide key evidence for the research and prevention of periodontal diseases. In addition, fluorescence spectroscopy can be used to evaluate the effectiveness of antibacterial treatments by monitoring the changes in biofilm activity to determine whether the treatment is effective.

#### **4.3. Application of Near-Infrared Spectroscopy in the Research of Periodontal Diseases**

Fluorescence spectroscopy and near-infrared spectroscopy each have their own advantages in the research of periodontal diseases. The former, based on fluorescence properties, enables highly sensitive and specific detection of biomolecules through probe labeling. The latter, by utilizing light absorption and scattering, can quickly obtain information on the molecular structures and chemical compositions of samples without the need for labeling. The combination of microscopic detection by fluorescence spectroscopy and macroscopic analysis by near-infrared spectroscopy complements each other, jointly facilitating the analysis of the mechanisms of periodontal diseases and precise diagnosis and treatment. Near-infrared spectroscopy can accurately identify the structural changes and concentration fluctuations of biomolecules such as proteins, lipids, and nucleic acids in periodontal tissues according to the unique vibration absorption characteristics of different molecules. By detecting the characteristic

absorption peaks of protein amide bonds, lipid hydrocarbon chains, and nucleic acid phosphate groups in the near-infrared band, it can reflect the pathological changes at the molecular level during periodontal lesions, providing key clues for in-depth analysis of the pathogenesis, disease monitoring, and diagnosis of periodontal diseases[10]. The applications of near-infrared spectroscopy are mainly reflected in the following aspects: In scientific research analysis, Zhai Yifan et al. used VOSviewer and CiteSpace to conduct a visual analysis of relevant literature in the past decade. By sorting out research hotspots and frontiers, it helps researchers quickly understand the trends in the field and provides guidance for subsequent experimental design, technical optimization, and other application directions[12]. In the field of disease detection, Guo Yuan et al. applied this technology to detect the blood oxygen content in the gingival tissues of periodontitis patients. By analyzing the spectral absorption characteristics of oxyhemoglobin (HbO<sub>2</sub>) and deoxyhemoglobin (Hb), they achieved an assessment of the hemodynamic changes in periodontal tissues. The research showed that the contents of HbO<sub>2</sub> and Hb in the periodontal pockets of patients with severe periodontitis were significantly higher than those in the healthy group. This achievement provides important scientific evidence for the precise diagnosis, treatment plan formulation, and efficacy monitoring of periodontitis[13]. Beyer-Hans K M C et al. studied the potential of infrared attenuated total reflection (IR - ATR) spectroscopy to detect the differences in the components of saliva supernatant between non-periodontitis patients and patients with generalized aggressive periodontitis. This technology can be used for the diagnosis of periodontitis and other potential periodontal diseases and has the potential for miniaturization, which can be used as a non-invasive screening test[14]. Liu K Z studied the use of near-infrared spectroscopy to monitor and determine the tissue oxygenation distribution in healthy and diseased sites of smokers and found that the spectral characteristics of the periodontal sites of smokers were usually similar to those of non-smokers[15].

## 5. SUMMARY AND PROSPECTS

As an emerging diagnostic and research tool, spectroscopic techniques not only improve the diagnostic accuracy and treatment effectiveness of periodontal diseases but also provide powerful technical support for a deeper understanding of the pathological mechanisms of periodontal diseases. They have shown broad application prospects in the diagnosis, treatment, and research of periodontal diseases. Combining them with artificial intelligence, nanotechnology, and multi-omics analysis is expected to further promote the precise diagnosis and personalized treatment of periodontal diseases. In the future, with the in-depth development of the concept of precision medicine, spectroscopic techniques are expected to become important tools in the clinical practice and basic research of periodontal diseases, providing more efficient and precise treatment for patients with periodontal diseases.

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