

Application of Raman spectroscopy in dentistry: current state of knowledge, future developments and clinical potential

Zastosowanie spektroskopii Ramana w stomatologii: aktualny stan wiedzy, kierunki rozwoju i potencjał kliniczny

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Abstract

Raman spectroscopy (RS) is an advanced optical technique that enables rapid, non-invasive, and highly specific chemical analysis of tissues and materials at the molecular level. In dentistry, RS shows significant clinical promise across various fields, including diagnostics, treatment monitoring, biomaterial assessment, and oral oncology. The technique allows for early detection of caries, real-time evaluation of biofilm, and monitoring of tissue regeneration processes after surgical interventions. Additionally, RS plays a crucial role in orthodontics, prosthodontics, and periodontology by offering insights into changes in mineral content, collagen structure, and interactions at the material–tissue interface. Despite challenges such as signal weakness, fluorescence interference, and cost, advances in RS technologies—including signal amplification methods and portable devices—are

expanding its accessibility and clinical utility. Integration with artificial intelligence and multimodal imaging techniques further enhances the diagnostic potential of RS. This review outlines the current state of knowledge on RS in dentistry, discusses technological developments, and highlights its future clinical applications.

Keywords: Raman spectroscopy, dentistry, diagnostic techniques, oral diseases

Streszczenie

Spektroskopia Ramana (RS) jest zaawansowaną techniką optyczną, umożliwiającą szybką, nieinwazyjną i wysoce specyficzną analizę chemiczną tkanek oraz materiałów na poziomie molekularnym. W stomatologii RS wykazuje duży potencjał kliniczny w różnych obszarach, takich jak diagnostyka, monitorowanie leczenia, ocena biomateriałów oraz onkologia jamy ustnej. Technika ta pozwala na wczesne wykrywanie zmian próchnicowych, bieżącą ocenę biofilmu bakteryjnego oraz monitorowanie procesów regeneracji tkanek po interwencjach chirurgicznych. Dodatkowo RS odgrywa istotną rolę w ortodoncji, protetyce i periodontologii, dostarczając informacji na temat zmian w zawartości minerałów, strukturze kolagenu oraz interakcji na granicy materiał–tkanka. Pomimo pewnych ograniczeń, takich jak niska intensywność sygnału, interferencje fluorescencyjne oraz wysokie koszty, rozwój technologii RS – w tym metod wzmacniania sygnału oraz urządzeń przenośnych – zwiększa jej dostępność i przydatność kliniczną. Integracja z algorytmami sztucznej inteligencji oraz technikami obrazowania multimodalnego dodatkowo wzmacnia potencjał diagnostyczny RS. Niniejszy przegląd przedstawia aktualny stan wiedzy na temat zastosowania RS w stomatologii, omawia najnowsze osiągnięcia technologiczne oraz wskazuje potencjalne kierunki jej przyszłych zastosowań klinicznych.

Słowa kluczowe: Spektroskopia Ramana, stomatologia, techniki diagnostyczne, choroby jamy ustnej

Introduction

In recent years, the rapid development of high-tech diagnostic methods has enabled the accurate and non-invasive characterisation of biological structures in dentistry. Among the technologies described, Raman spectroscopy (RS) is emerging as a particularly promising tool, whose main advantage is that it provides precise information, even at the single molecule level, without the need for time-consuming sample preparation or contrast agents. This method, based on the principle of inelastic light scattering, allows the detection of frequency changes corresponding to specific vibra-

tional modes of molecules, making it possible to obtain a molecular ‘fingerprint’ of the analysed material [1].

RS is a non-destructive technique, meaning that it does not damage or modify the sample under investigation, while at the same time showing high specificity to the materials being examined in their natural, unaltered state. With adequate sample hydration, this method can outperform traditional imaging techniques and classical chemical analyses by providing more detailed and selective data. RS is widely used in many areas of medicine, including oncology, research into the implementation of novel therapies, or monitoring disease progression [2, 3].

Despite its many advantages, spontaneous Raman scattering is characterised by very low intensity. Consequently, a number of techniques have been developed to enhance the Raman signal. Among the most important of these are: Stimulated Raman Scattering (SRS), Coherent Anti-Stokes Raman Scattering (CARS), and Surface-Enhanced Raman Spectroscopy (SERS), Tip Enhanced Raman Scattering (TERS) and Spatial Offset Raman Spectroscopy (SORS). The development of RS techniques towards the aforementioned methods has made it possible to significantly increase the sensitivity of measurements through multiple signal enhancement (even by several orders of magnitude) and the analysis of deeper tissue layers [2, 3, 4]. Thanks to such properties, these techniques are particularly applicable in medical diagnostics and biological research.

RS has gained considerable interest in dentistry because it can be used both as a diagnostic tool in clinical practice and for the analysis of dental materials. One of its greatest advantages is its ability to detect early carious lesions by analysing changes in the mineral content of enamel and dentin. Unlike other diagnostic methods, which are mainly based on classical imaging techniques, RS allows the identification of demineralisation processes at the molecular level, i.e. even before they become visible on imaging examinations, which allows early intervention, as well as the use of much more effective prophylactic methods [5, 6]. In addition to its use in caries detection, RS is also used for monitoring the effectiveness of treatment with fluoride therapeutic agents. This method enables an objective evaluation of enamel remineralisation by assessing both the extent of mineral recovery and the dynamics of chemical and structural changes in tooth tissue, in relation to the intended biological and chemical effects. As a result, it serves as a reliable tool for determining the suitability of a chosen therapeutic approach [7]. Another area of RS use in dentistry is the assessment of defects in the developing enamel, such as fluorosis or insufficient mineralisation of molars and incisors. RS is also used to analyse interac-

tions between dentin and adhesive materials during restorative and prosthetic methods [8, 9]. Advances in the use of Raman imaging and the development of fibre-optic probes are significantly facilitating the use of RS for real-time *in vivo* diagnostics, opening up new possibilities for the development of minimally invasive tissue examinations, assessment during surgical cuts, and conservative treatment. Due to its ability to non-invasively monitor the cellular and extracellular matrix, as well as provide real-time biochemical data, RS provides important support for translational research and the development of innovative clinical solutions, including stem cell-based therapies [3].

Given the variety of possibilities for use and the growing body of evidence supporting the validity of RS in dentistry, the technique can be seen as a versatile, informative, and patient-friendly diagnostic tool. Its implementation into clinical work offers opportunities not only for early lesion detection and treatment monitoring, but also for accelerating the development of research into new biomedical materials that could be used to treat dental patients. The aim of this article is to discuss the main areas of application of RS in dentistry, taking into account the current state of knowledge, development prospects, and potential clinical benefits of its implementation.

Raman spectroscopy - fundamentals and technological developments

Physical basis of Raman spectroscopy

RS is based on phenomena in quantum optics, in particular changes in the polarizability of atoms, i.e. their ability to modify the electron cloud distribution under the influence of an external electric field. In the case of RS, the source of such a field is a photon emitted by a laser. When the polarizability of an atom is altered by the nuclear motion of the molecule, Raman scattering occurs, which forms the basis of spectral analysis in this technique. The polarization of the molecule allows a change in the scattering state, i.e. a change in the energy of the photon emitted by the laser [10]. Raman scattering is sometimes referred to as two-photon scattering because it involves a photon incident on the sample under investigation (coming from the laser) and a scattered photon, which has a different energy after interacting with the sample molecule. The energy difference between these photons is expressed as a Raman shift, given in wave numbers, i.e. in units corresponding to the inverse of the wavelength measured in centimetres (cm^{-1}). This value is the basis for the generation of Raman spectra and is a key parameter used in their analysis and interpretation [11]. RS also allows the concentration of a substance in a sample to be quantified. Each wave number corresponds to a characteristic vibration of a specific chemical bond

in a molecule, and the intensity of the spectral band reflects the amount of that substance. The combination of band positions and intensities creates a unique pattern, called the molecular ‘fingerprint’ of the molecule.

When the polarizability of the molecules remains constant during their oscillation, only an elastic scattering spectrum, called the Rayleigh spectrum, is observed. This spectrum forms the background for the Raman scattering signal recorded on the surface of the sample under investigation. Although it is a ‘background’ phenomenon, the Rayleigh spectrum can also provide valuable information about the physical properties of the structure under analysis, such as homogeneity or surface features [12].

How are Raman spectra interpreted for biological samples?

Raman spectra of biological samples allow the identification of characteristic vibrational models of molecules, including proteins, nucleic acids, lipids, and other cellular components, which exhibit unique spectral features depending on the type of chemical bonds and molecular structure [13]. The properties (features) of a given sample, which are visible in the Raman spectrum, make it possible to distinguish between healthy and pathologically altered tissues and provide information about molecular changes associated with the development of pathological processes, such as tumorigenesis or bacterial infections [14].

What types of Raman spectrometers are used in biomedical or dental research?

Various Raman spectrometers using more than 25 different RS techniques, such as spontaneous Raman scattering, hyper-Raman scattering, Fourier transform Raman scattering (FT-Raman), Raman-induced Kerr effect spectroscopy (RIKE), and stimulated and coherent Raman scattering (SRS/CARS), are used in biomedical and dental research [4]. In addition to full-size laboratory devices, portable models are also available that enable the use of this technology both in the clinical setting and in the daily practice of the dental office - for example, for rapid diagnosis of lesions or monitoring of treatment progress [13]. These devices enable the detection of specific molecular structures in complex biological matrices, offering real-time diagnostics with minimal sample preparation [13].

Recent technological advances in miniaturisation include the development of portable and handheld Raman spectroscopes that can be used for real-time diagnostics in clinical settings [15]. These devices are compatible with artificial intelligence-based data processing, which significantly improves the accuracy and speed of diagnostic analyses

[16]. The use of SERS substrates in miniaturised devices further increases the sensitivity of analyses of complex biochemical structures [13]. With these innovations, Raman spectroscopes are becoming an increasingly versatile and effective tool for rapid and precise medical diagnostics, including in the field of dentistry.

What is SERS and what is its application in medicine?

As mentioned above, SERS is one of the advanced spectroscopic methods that significantly enhance the intensity of Raman signals, which tend to be very weak under standard conditions. This enhancement is made possible by the phenomenon of surface plasmon resonance, occurring on nanostructured metallic surfaces, usually made of precious metals such as gold or silver. These nanostructures cause a local concentration of the electromagnetic field, leading to stronger light scattering by the molecules under study [17]. In medical diagnostics, the SERS technique shows great potential for the early detection of cancer through the identification of tumour markers and is also used for the rapid detection of pathogens such as *Staphylococcus aureus* bacteria and respiratory viruses, including influenza viruses and coronaviruses [17, 18]. In addition, the SERS technique facilitates quantitative analysis of biomarkers in body fluids such as saliva and blood, thereby supporting the development of personalised medicine and facilitating rapid therapeutic decision-making [13].

Clinical applications across multiple dental specialties

Raman spectroscopy in restorative dentistry: caries detection and endodontics

Detection and monitoring of early carious lesions

One of the most promising applications of RS in restorative dentistry is its ability to detect early carious lesions that are not usually seen during probing with a dental explorer. RS makes it possible to identify subtle biochemical changes in both enamel and dentin. This technique allows changes in mineral or salt content, such as phosphates or carbonates, to be monitored, providing the opportunity to recognise early demineralisation processes that are characteristic of the onset of caries formation. Although characteristic band positions corresponding to phosphates (approximately 960 cm^{-1}) and carbonates (approximately 1070 cm^{-1}) have been described in the literature, the exact spectral signatures depend on tissue structure and measurement conditions [6]. These changes appear much earlier than the defects visible on X-rays, allowing rapid preventive measures to be taken.

Other advanced techniques, such as polarised RS, enhance diagnostic sensitivity by quantifying the depolarisation coefficient in enamel. This parameter allows the creation of a

molecular ‘fingerprint’ that distinguishes with high specificity between healthy tissue and demineralised areas, allowing clinicians to tailor preventive methods or remineralisation therapies to individual patients [5].

RS can also be used to monitor treatment effects. Tracking the intensity of phosphate bands during remineralisation treatment with fluoride or calcium allows for accurate determination of the degree of remineralisation in the enamel, thus supporting preventive interventions [19].

Use of RS in endodontics

In addition to detecting carious lesions, RS is also becoming increasingly important in endodontics. As mentioned earlier, the technique detects subtle changes at the pulp-dentin interface, making it an extremely valuable tool in assessing the health of the tooth pulp. For example, specific changes in amide bands I and III, as well as collagen-related signatures, can indicate the presence of inflammation, necrosis, or regenerative processes in the pulp [20].

RS is also an effective method in the evaluation of endodontic root canal cleaning with chemicals. By detecting smear layers, bacterial metabolites, as well as mineralised debris on the dentin surface, RS significantly improves the evaluation of root canal debridement efficiency, a key step that determines the success of endodontic treatment [21].

Furthermore, RS facilitates the evaluation of biomaterials used in endodontics, such as sealants, cements, or regeneration carriers. By evaluating biomaterials, it is possible to confirm whether a material adequately integrates with dentin and whether it exhibits adequate stability and biocompatibility in the dental root environment. Studies indicate that RS can also be used to monitor the polymerisation process, detect degradation, and allow assessment of the bonding of materials to surrounding tissues, providing a more comprehensive understanding of the determinants of long-term treatment efficacy [22].

In summary, RS offers a breakthrough solution in restorative dentistry by allowing the detection of early carious lesions that are not visible to the naked eye, the monitoring of remineralisation efficiency, and the evaluation of endodontic treatment at the molecular level. Thanks to its non-invasive nature and high specificity, RS has gained the potential to become an indispensable tool in modern dentistry. Ongoing and dynamic technological advances, such as the integration of fibre-optic probes and artificial intelligence, are facilitating the broader adoption of this method in clinical practice.

Raman spectroscopy in periodontology and biofilm assessment

Assessment of biofilm

By offering non-invasive and specific capabilities for the assessment of bacterial biofilms and the tissues covered by them, RS has also become a significant tool in periodontology. The sensitivity of RS at the molecular level allows for the accurate characterisation of the complex biochemical processes associated with periodontal disease, including biofilm maturation, tissue inflammatory processes, or alveolar damage.

Examination of the microbial layers in RS allows for the identification of their biochemical components, such as lipids, proteins, and polysaccharides, which change with the time of biofilm formation. Changes at the molecular level are an indicator of the physiological maturation process of biofilms, which runs in parallel with an increase in their structural complexity and increasing pathogenic potential [23, 24].

Analysis of the extracellular matrix and potential targets for antimicrobial therapy

RS also provides a useful tool in the assessment of extracellular polymeric substances (EPS) that form the biofilm matrix. Spectral analysis enables the assessment of polysaccharide signatures present in EPS, which are essential for the stabilisation and maintenance of biofilm structure. The acquisition of this information enables the rapid development of targeted antimicrobial strategies [25, 26].

Detection of inflammatory lesions in the gums

Clinical studies demonstrate that RS can be used as an important method to detect inflammatory changes in the gingiva and alveolar processes. The ability of RS to detect biochemical changes, such as changes in the concentrations of substances in the gingival fluid, enables the objective assessment of the course of periodontal disease [27].

Monitoring changes in the alveolar processes

The progression of periodontal disease is often asymptomatic, which is the case, for example, with alveolar bone loss, occurring long before obvious clinical changes appear. In such cases, RS offers a unique opportunity for early detection of biochemical changes by identifying subtle differences in the mineral composition and structure of bone-building proteins. Under inflammatory conditions (e.g. in periodontitis), osteoclast activity leads to a reduction in bone mineral density and disruption of collagen cross-linking - both of which can be identified with RS through characteristic changes in the recorded spectra [28]. It is worth emphasising once again

that such assessments can be carried out entirely non-invasively, without harming surrounding tissues and without the need for in vivo tracers. Such possibilities offer new perspectives for the long-term monitoring of bone remodelling processes in patients undergoing periodontal treatment, or who are in clinical trials that focus on the evaluation of new restorative materials. The ability to track changes in bone composition and structure at the molecular level - rather than the classic assessment of bone volume or density - opens up new possibilities as to how to assess treatment efficacy or disease progression.

Assessment of collagen structure and tissue cohesion

Collagen is the structural backbone of both gingival and alveolar connective tissue, and its degradation is one of the hallmarks of periodontitis. While standard diagnostic tools do not highlight clinical signs of collagen damage until specific structures are damaged, RS - by analysing vibration bands corresponding to amide I and III regions in Raman spectra - allows detection of early damage because these bands change their position and intensity as changes and modifications to the bonds present in collagen structures develop. As mentioned earlier, this phenomenon represents a kind of molecular 'fingerprint' relating to tissue integrity [29].

Such a high sensitivity of the test allows clinicians and researchers to monitor tissue healing processes after periodontal treatment, including surgical procedures or biomaterial implantation, and also enables comparative analysis in relation to other therapeutic methods that affect connective tissue at the molecular level. Indeed, RS makes it possible to observe 'latent' regeneration processes taking place beneath the tissue surface, which are invisible to the naked eye, and their detection plays a key role in obtaining beneficial and long-term treatment results.

Technological advances and clinical integration

The aforementioned SERS and SORS techniques, have further broadened the scope of analysis in periodontology by significantly increasing the sensitivity of the examination and enabling the assessment of deeper tissue layers. These advances open up new possibilities for their direct application in chairside settings, enabling real-time diagnosis and monitoring of periodontal disease treatment [30, 31].

Raman spectroscopy in orthodontics: force-induced tissue changes

Effect of orthodontic forces on biochemistry in enamel

The tooth movement caused by the presence of braces exerts continuous forces on the hard tissues of the teeth, including the enamel, leading to disruption of their structural and

biochemical stability. Studies using RS have shown that mechanical loading can cause microdamage in enamel, as evidenced by changes in phosphate and carbonate bands - key markers of mineral content [32]. Abnormalities in the amount and ratio of individual minerals in the enamel predispose to increased demineralisation and an increased risk of caries development during orthodontic treatment [33].

Cement remodelling and root resorption

The dental cementum, which covers the tooth root and plays an essential role in its fixation in the alveolus, adapts the teeth to the forces generated by orthodontic treatment. Excessive orthodontic forces lead to dysregulation of the processes involved in bone remodelling, which can result in excessive external root resorption, as well as long-term tooth instability [34]. RS allows the detection of changes related to cementum remodelling, including the assessment of the activity of the RANK ligand (Receptor Activator for Nuclear Factor κ B Ligand, RANK), which is involved in the activation of osteoclasts involved in root resorption [35].

Alveolar bone remodelling and mechanical force dynamics

Tooth movement induced by orthodontic treatment is based on bone remodelling. In studies using RS, it has been shown that the technique used makes it possible to track changes occurring in response to mechanical stress, including those relating to bone mineral density, as well as collagen structure. Such changes can be detected by analysing the observed Raman bands, which correspond to phosphate and amide groups that correlate with mineralisation levels, as well as collagen integrity [36, 37]. Raman spectra make it possible to determine whether mineralisation or resorption is occurring at a particular site, while Raman mapping provides detailed information regarding the location and intensity of these processes in individual teeth, as well as their joint response to orthodontic forces [38].

Monitoring root resorption with molecular precision

One of the most worrying iatrogenic phenomena accompanying orthodontic treatment is the process of dental root resorption. RS enables real-time, non-invasive detection of early loss of minerals found in both dentin and dental cementum. By assessing the decrease in the intensity of the Raman bands characteristic of hydroxyapatite, as well as changes in the ratio of mineral to organic matrix, it has become possible to quantitatively detect even subclinical resorptive changes [39], which influence the modification of treatment strategies and reduces the risk of irreversible damage.

Clinical relevance and prospects for implementation

The potential for the application of RS in orthodontics is reflected in the possibility of personalised monitoring of treatment progress. The ability to detect the smallest changes at the molecular level long before they become clinically apparent allows for proactive adjustment of the use of orthodontic forces, as well as precise treatment planning [40, 41]. Advancements in modern technologies, including the miniaturisation of RS systems and fiber-optic probes, are creating realistic opportunities for their integration into routine dental practice - supporting both diagnosis and real-time treatment monitoring.

Diagnosis of neoplastic lesions and oral pathology

Molecular profiling and tissue differentiation

In terms of sensitivity and precision in the diagnosis of neoplastic lesions, RS shows significant advantages over classical diagnostic methods based on histopathology, biomarker testing, *vital staining*, DNA analysis, biopsy, and optical techniques. The use of RS to detect early dysplastic, or neoplastic lesions of the oral cavity utilises the identification of changes in metabolism and cellular structure that are characteristic of neoplastic transformation [42]. Tissues within the oral cavity affected by malignancy, particularly in the case of squamous cell carcinoma, show distinct changes in Raman spectra, e.g. by changing the intensity and shifting of bands corresponding to specific biological molecules (nucleic acids, lipids and proteins), reflecting pathological changes at the molecular level, with the sensitivity of such an examination for altered tissues reaching up to 97% [43]. The specific spectra of the Raman spectrum make it possible to distinguish precisely between cancer cells and healthy tissue. It has been recognised that spectra with a clear predominance of lipid signals are characteristic of healthy tissue, whereas a predominance of protein and nucleic acid features in the spectrum is characteristic of the neoplastic process [44]. However, diagnostic algorithms based only on the lipid-to-protein ratio might fail to distinguish variations in tissue morphology, for example, differences in epithelial thickness, particularly in cases without neoplastic lesions. For this reason, in order to develop a diagnostic model with high specificity and reproducibility, a database containing histopathologically classified reference from RS of the oral mucosa and its lesions has been proposed [45]. In order to increase the comparability of results between studies, it may be helpful to introduce standardised indices and uniform criteria for the analysis of spectroscopic data. For example, three indices relating to phenylalanine (phenylalanine index, PhI), protein (protein index, PI) and lipid (lipid index, LI), respectively, were developed

based on the mean values of the ratios between the areas of the bands corresponding to phenylalanine, amide groups I and III and CH groups. The values of PI and PhI indices were found to be significantly higher in tumour tissues than in healthy mucosa. An inverse relationship was observed for LI [46].

Saliva composition testing and non-invasive diagnostics

In addition to examining tissues, RS can also be used to study body fluids, including saliva composition in relation to potential dysplastic and neoplastic lesions. In saliva samples from patients diagnosed with oral squamous cell carcinoma, significant differences in the vibrational characteristics of biomolecules are observed - most commonly concerning bands corresponding to amide groups I and III of proteins, as well as lactates, lipids, and antioxidant compounds - which clearly distinguish these samples from the saliva of healthy individuals [47]. However, obtaining an accurate and reproducible analysis/mapping of the spectral profile of saliva for each type of sample depends on a number of factors, including the collection methodology (passive vs. stimulated method), the preparation of saliva for testing (in the physical solid vs. liquid state), or the use of a particular type of RS technology (e.g. conventional RS vs. SERS). For this reason, literature data on the sensitivity and specificity of Raman analysis of saliva show significant discrepancies, indicating the need for a consistent and reproducible methodology [48].

Intraoperative use and evaluation of incision margins

Assessment of surgical margins is an important prognostic factor in oral cancer surgery. The use of a RS-based objective intraoperative assessment of resection margins (Raman Intraoperative Assessment of Resection Margins, RIOARM) device, which utilises a range of high wavenumbers in the Raman spectrum, provides the opportunity to objectify and safely perform intraoperative margin assessment by offering an immediate and non-invasive method for intraoperative tissue examination [49]. This use of real-time RIOARM improves surgical outcomes and also reduces the risk of recurrence.

Integration with endoscopy and optical biopsy

In recent years, the possibility of using RS as a tool to support the development of optical molecular diagnostics has been increasingly raised. The development of fiber-optic RS technology, suitable for routine, highly sensitive, and non-invasive diagnostics, opens up new *in vivo* diagnostic possibilities in the most inaccessible areas of the oral cavity and

the oropharynx. To achieve the highest sensitivity and specificity, it is recommended to use two wavelength ranges in Raman analysis. The first is the ‘fingerprint’ region ($400\text{--}1800\text{ cm}^{-1}$), which enables identification of specific chemical compounds and differentiation between healthy and dysplastic tissue. The second is the high-wavenumber region ($2800\text{--}3200\text{ cm}^{-1}$), which, although providing less detailed information, is more resistant to interference and better suited for rapid clinical analysis, allowing for immediate decision-making at the patient’s side [50]. The dynamic development of the technology allows the construction of compact RS-based devices, which is an important step towards its implementation in everyday clinical practice. However, there is still a lack of solutions that are attractively priced and available on a wider scale.

Application of artificial intelligence algorithms and spectral classification

The use of artificial intelligence (AI) algorithms, including machine learning, is finding increasing application in the creation and analysis of medical databases, supporting diagnostic, prognostic, and treatment personalisation processes. Research in recent years has increasingly combined RS with advanced multivariate analysis and machine learning techniques to improve diagnostic accuracy. Algorithms based on Raman signatures enable precise differentiation of healthy, dysplastic and cancerous tissues. Moreover, they can effectively distinguish squamous cell carcinoma from adenocarcinomas when classifying neoplastic lesions [51]. Such models significantly support the development of fully automated diagnostic devices for clinical use.

Raman spectroscopy in prosthetics

Ceramic materials

Ceramic materials are widely used in dental prosthodontics due to their aesthetics and high biocompatibility. The properties of ceramic materials after deposition in the oral cavity are highly dependent on their microstructure and phase composition, which vary depending on the manufacturing technology and the aging processes occurring in the oral environment. In this context, RS represents an extremely promising diagnostic tool for the detailed evaluation of these parameters. Thanks to its high spectral resolution, it allows the analysis of crystallinity, the identification of characteristic chemical bonds, and the evaluation of lattice vibrations, providing valuable information at the material structure level [52].

RS allows the identification of specific vibration bands associated with ceramic components (e.g. zirconium oxide, alumina), as well as silicate-based materials. Differences in

peak positions and intensities may indicate phase transitions, structural inhomogeneities, or the accumulation of stresses arising both during the manufacturing processes and after the material has been deposited in the oral cavity [53]. Observation and analysis of RS spectral signatures enable optimisation of manufacturing processes and allows effective quality control of materials prior to their use in the clinic.

Resin-based composites and adhesive systems: molecular monitoring of polymers

Resin-based composites, including adhesive materials and polymers used in the placement of prostheses, play a key role in both temporary and permanent prosthetic restorations. RS provides precise monitoring of the materials by analysing their polymeric structure, especially during and after the curing process, through the detection of C=C bonds. These are used to calculate the degree of monomer conversion, which directly correlates with material strength, wear and degradation resistance, or marginal integrity [54, 55].

In addition, Raman analysis makes it possible to assess changes related to the composition of fillers, cross-linking agents and reinforcing substances, among other things. For example, the addition of nanomaterials containing silver or boron nitride causes characteristic changes in the Raman spectrum, which are indicative of the formation of new chemical bonds or molecular structures in the resin matrix [56]. RS allows better quality control of materials used in prosthetics, improvement of their formulation, and prediction of their behaviour under clinical conditions. As a result, this translates into increased efficacy and durability of prosthetic restorations. The ability to detect subtle structural changes is crucial for assessing bifunctionality and introducing next-generation materials.

Degradation and ageing: long-term monitoring of prosthetic materials

Prosthetic materials, when exposed continuously to the complex conditions of the oral cavity, naturally undergo gradual degradation. Aging processes such as hydrolysis, oxidation, or thermal fatigue - the gradual material damage caused by repeated temperature fluctuations in the oral cavity - induce internal stresses within the material's structure. Over time, these stresses result in micro-cracking and a deterioration of the material's mechanical properties, often occurring well before any visible damage becomes apparent. RS allows early detection of such changes by identifying specific degradation products, including the appearance of carbonyl and sulphonyl groups in polymeric materials, as well as crystalline phase transformations in ceramic materials [57]. RS also makes it possible to compare the properties of materials under simulated aging conditions, i.e. laboratory conditions in which the material is exposed to simulated thermal cycles, artificial saliva or distilled water (effect of hydro-

lysis), UV radiation (oxidation), chemicals (substances of different pH that may be present in the diet), or mechanical loads [58]. By using this diagnostic technique, it is possible to optimise the selection of durable prosthetic materials used in clinical practice.

Interaction with saliva and surface reactivity

A key factor affecting the stability of prosthetic materials is the phase boundary between the material itself and saliva, especially in the case of removable prostheses. Saliva, which includes, in addition to water, enzymes, proteins and ions, can interact with the surface of the materials, promoting adsorption, and the deposition of various substances, which can affect the mechanical and aesthetic properties of the prosthesis. RS is sometimes used to study such interactions, highlighting how specific saliva components attach to resin-based composites or ceramic materials, and whether they cause changes in the biochemical profile of the material under study or initiate early biofilm deposition [59]. Understanding these interactions early in the design and testing phases of prosthetic materials enables the development of composites that are more resistant to contamination, thereby improving both aesthetics and durability in clinical use.

Biofilm formation and biodegradation of prosthesis surfaces

Biofilm colonisation in prosthodontics is a huge challenge, especially in implant-supported prostheses. Once a biofilm has formed, it not only worsens overall oral hygiene, but also contributes to material degradation through acid production, enzymatic activity, and mechanical erosion.

RS allows a detailed, non-invasive analysis of the biofilm developing on the surface of prostheses, providing information regarding the presence and status of bacterial metabolites, mineralisation activity, and extracellular matrix composition. By comparing spectra obtained at different time intervals for different materials, researchers can determine which denture surface is most susceptible to biofilm-induced degradation [60]. This facilitates the more effective design of prosthetic materials that are better resistant to microbial colonisation.

Raman spectroscopy in dental surgery: monitoring healing processes, supporting reconstruction and preventing complications

Assessment of wound healing after tooth extraction

Every tooth extraction initiates a complex cascade of biological processes consisting of inflammation, tissue remodelling and regeneration. RS, by non-invasively tracking changes

in lipid and protein composition, as well as collagen structure and content, can be applied to monitor the healing process of post-extraction wounds. As is well known, collagen plays a key role in tissue regeneration. In this context, the observed changes in the intensity of amide I and III bands, which may indicate collagen maturation and collagen fiber remodelling, are important, as mentioned earlier. In turn, shifts in lipid-related bands may reflect increased inflammation or bacterial colonisation within the wound [61]. The use of RS to assess wound healing primarily focuses on evaluating the extent of tissue regeneration and can indirectly support therapeutic decisions - such as determining the optimal timing for suture removal or the necessity of adjunctive treatments in cases of impaired healing. The ability to non-invasively track biochemical changes within the wound makes this technique a promising tool to support treatment and individualise post-extraction care.

Assessment of soft tissue and bone regeneration

In cases requiring bone grafting or soft tissue transplantation, RS can be used to assess the biochemical quality of the regenerated tissues. Analysis of bone mineral content and assessment of the collagen profile of the connective tissue allows assessment of the regularity of the healing process and the effectiveness of tissue integration, as confirmed by results obtained in animal models [62]. This type of information may be particularly important in assessing the course of complex surgical procedures such as assisted bone regeneration or flap reconstructions, where even slight changes in biochemical signals may indicate the risk of graft rejection or delayed tissue healing.

Material-tissue interactions in reconstructive surgery

In recent years, an increasing number of studies on animal models have utilized RS for the biochemical evaluation of biomaterials used in maxillofacial reconstruction. Regardless of the type of material tested - whether titanium implants, bone substitutes, or collagen membranes - RS enables the detection of inflammation and degradation processes. Spectral shifts related to calcium phosphate bands and collagen cross-links are particularly important for assessing biocompatibility and the biological response of the organism [63].

Raman spectroscopy in everyday clinical practice - possibilities and limitations

Advantages: precision, versatility and clinical potential

Undoubtedly, the most important advantages of the use of RS in dentistry include the possibility of non-invasive and rapid chemical analysis of tissues and materials, high sensi-

tivity in detecting biochemical changes at the molecular level, the ability to differentiate between healthy and pathological structures, the lack of need for tracers or dyes, and the possibility of in vivo application, which favors the monitoring of disease processes and healing [64]. It is also noteworthy that RS allows real-time assessment of oral tissues, making it particularly useful for both prophylaxis and monitoring the patient's condition after surgical procedures. An additional advantage is its exceptional versatility - Raman spectra can be obtained from various sources, such as enamel, dentin, pulp, dental materials, or even saliva. Such a broad spectrum of RS applications supports the development of modern diagnostic methods in many areas of dentistry, including periodontology, dental surgery, prosthodontics, and oral oncology [65].

One of the most promising aspects is the integration of RS with artificial intelligence and multimodal imaging methods. Machine learning algorithms allow rapid and automatic tissue classification, even under difficult conditions. Combining RS with techniques such as optical coherence tomography, endoscopy, or fluorescence microscopy provides high biochemical specificity and precise localisation of lesions, which can significantly reduce the need for classical biopsy [66].

Limitations: signal strength, fluorescence, cost and availability

Despite the numerous advantages offered by this technique, it is essential to acknowledge that certain limitations remain. One of the main challenges of this method is the limited signal efficiency, which means that only a small fraction of photons undergo Raman scattering, so that obtaining a spectrum requires longer exposure times and can be prone to interference. For this reason, intensive efforts are underway to improve light sources, detectors, and data processing methods to make RS more accessible in everyday clinical practice. This includes the development of advanced techniques - for example SERS, CARS, TERS, and SORS, which significantly enhance signal strength, sensitivity, and the analytical capabilities of the method [30, 4].

Another important limitation is fluorescence interference. Biological tissues naturally contain fluorophores, such as porphyrins or flavins, which emit intense fluorescence under laser excitation. This phenomenon can significantly hinder and, in some cases, even completely mask the Raman signal, especially in inflamed or cancerous tissues. However, there are effective strategies to reduce this effect. These include the use of longer excitation wavelengths (e.g. 785 nm or 1064 nm), changing excitation conditions and digital processing of the spectral background to reduce interference [67].

It is clear that using highly advanced technology requires significant financial investment. Equipment related to RS, such as cooled CCD detectors, lasers, and precision optics, can incur costs ranging from tens to hundreds of thousands of dollars. This substantial financial investment poses a significant barrier to the widespread adoption of RS technology in both clinical and research settings. Additional costs arise from the need for staff training, as well as ongoing system maintenance and calibration. To address these challenges, efforts are underway to develop more compact and portable RS systems that could be utilized not only in research laboratories but also in private dental practices and clinics.

Summary: clinical potential of Raman spectroscopy in dentistry

RS represents a promising diagnostic tool in dentistry, enabling non-invasive, real-time molecular analysis of tissues and materials. The method allows early detection of carious lesions, assessment of biofilm, orthodontic and prosthetic materials, detection of dysplastic lesions, and monitoring of healing and tissue regeneration processes. Through integration with artificial intelligence algorithms and multimodal imaging techniques, RS is gaining precision and automation, which can reduce the need for classical invasive biopsy.

Despite limitations such as fluorescence interference, weak signals, and high costs of equipment and training, the development of advanced signal amplification techniques, along with efforts to create compact devices and reduce testing expenses, supports the potential integration of RS into routine clinical practice.

In summary, RS offers high specificity and sensitivity, with the potential to revolutionize dental diagnostics, pending further technological optimization and clinical validation.

The authors declare no conflict of interest.

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