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Role of infra red spectroscopy in oral medicine

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Abstract

In recent years, several prognostic markers have been used as indicators of disease progression in oncology. Accurate and reliable decision making in the spectroscopic diagnosis can help in the planning of suitable surgery, therapy and to improve patients monitoring through different stages of disease.

Use of spectroscopy for detection of cancer is more reliable as compared to any other techniques. We have implemented the IR spectroscopic techniques for the detection of oral diseases like oral pre cancerous lesions and oral cancer. Bio-medical image processing has been used to derive useful information from spectrums of data.

The objective of the present work is to improve the primitive methodology of distinguishing cancerous and non-cancerous images by just visual inspection so as to provide more information to the doctor and clinical treatment planning system.

Keywords: Infra red spectroscopy, oral diseases, oral medicine

Introduction

Medical image processing has been used to derive useful information from spectrums of medical data. Spectrophotometer is the instrument that determines the absorption spectrum for a compound. Infrared spectroscopy involves interaction of IR radiations with matter. A pattern produced by the design of the optical pathway is called interferogram.[1]

It is a complex signal but its wave-like pattern contains all the frequencies that make up the IR spectrum. A mathematical operation known as Fourier transform (FT) can separate the individual absorption frequencies from the interferogram producing spectrum. This type of instrument is FTIR.

The interferogram is subjected to a Fourier transform which yield the spectrum of the background and the compound. IR rays are non-destructive to biological samples. In India, oral cancer ranks number one in prevalence among all cancers in male patients and number three among cancers in female patients.[2]

It accounts for 16% of all female cancers and 22.9% of invasive cancers in women. 18.2% of all cancer deaths worldwide. Oral cancer is diagnosed by oral examination and palpation, usually performed by dentists or physicians. Visual inspection of the oral cavity is performed under normal white light illumination, followed by palpation of suspicious lesions. These are not of use due to their limited spatial resolutions, accuracies, and/or mobility.[3]

Hence, a new technique is needed for the detection of oral cancer. Spectroscopy is a technique of interaction between matter and radiated energy that can be used for the analysis of oral lesions. FTIR spectroscopy has been used for cancer detection in oral mucosa. The biomarkers of oral cancers include variation in protein, lipid, NADH and Collagen. The intrinsic fluorescence spectra was extracted from in vivo fluorescence spectra in the oral cavity with a mathematical model and NADH increases and collagen decreases with oral cancer progression was observed. [4]

Studies using Raman spectroscopy for diagnosis of oral cancer are reported and shown that lipids dominated normal oral epithelial tissue spectra while malignant tissues showed protein-dominated spectra. The normal and malignant oral tissue types in both retrospective as well as prospective studies based on large spectral data were also classified [6]. The performance of visual examination for oral cancer detection has been systematically reviewed. Thus, there remains an important need for alternative diagnostic methods that can enhance the visualization of oral lesions and particularly help discriminate benign and premalignant lesions.[5]

Infra red spectroscopy in oral medicine and diagnosis

Oral cancers, a subtype of head and neck cancers, are cancers of the oral cavity. Oral cavity represents the first structure of the aero digestive tract and is composed of distinct anatomic subsites. Lips, buccal mucosa, the upper and lower alveolar ridges with their attached gingiva, the retromolar trigone, the hard palate, the floor of the mouth, and the anterior two-thirds of the tongue majorly constitute the oral cavity.[6]

Oral cancers can arise in any of these subsites. In Western countries, tongue, floor of the mouth (FOM), and lip account for 70% of all cancers while in the Indian subcontinent, buccal mucosa and tongue along with the lip are the most commonly affected subsites. This disparity in most commonly affected subsites can be attributed to differences in ethnic, social, and lifestyle-related factors. Oral cancers are a major health problem worldwide, with an annual incidence estimate of approximately 275,000 cases.[7]

Oral cancers form a significant health burden in developing countries like India, where they account for over 30% of all cancers with 80,000 new cases reported each year. Tobacco (both smoking and smokeless) and alcohol are major etiological factors. Visual inspection, followed by biopsy and histopathology of suspicious lesions found during clinical examination, is the gold standard for diagnosis. Oral cancers are often preceded by clinically visible mucosal alterations termed **"**precancer stages**."** [8]

These precancer stages may refer to the presence of a benign lesion or morphologically altered tissue that has a greater than normal risk of malignant trans formation. Leukoplakia, erythroplakia, oral submucous fibrosis

(OSMF), tobacco pouch keratosis, and lichen planus are some forms of oral precancer or premalignant lesions. These lesions may or may not be dysplastic on histo pathological assessment. [9]

The removal of lesions with moderate or severe dysplasia is advocated while mild dysplasia is followed up for reversal or progression. Treatment for oral cancer includes surgery, radiotherapy, and chemotherapy; surgery combined with chemotherapy and radiotherapy improves overall survival. In spite of the advancement in surgical and treatment modalities, low disease-free survival rates have been observed for several decades. The main reasons for the dismal survival rates include - diagnosis mainly in advanced stages, recurrence, inadequate access to health services, and lack of primary knowledge about causative factors. It is known that early detection of oral cancer and recurrence can enhance survival rates and improve overall quality of life. Adjunct techniques such as tissue staining using toluidine blue, oral cytology, tissue fluorescence (VELscope), and chemiluminescence (ViziLite) based methods are being explored as comple mentary techniques for early diagnosis. [10]

While tissue staining involves the use of metachromatic dyes such as toluidine blue which have high affinity for DNA and can differentiate normal and abnormal tissues based on DNA content, oral cytology involves the collection of trans epithelial samples from oral mucosa and subsequent cytomorphometry to identify abnormal cells. Light-based methods such as VELscope and ViziLite employ native tissue characteristics such as fluorescence and reflectance to identify abnormal regions. Serum- and saliva-based molecular diagnostic markers are also being investigated.[11]

In recent times, optical spectroscopic approaches have also been explored for oral cancer diagnosis. Optical spectroscopy involves the study of light-tissue interaction. The optical spectrum derived from any tissue contains information about the histological and biochemical makeup of that tissue. Because of the accessibility of the oral cavity, there has been increasing interest in the use of fiber-optic probe coupled optical spectroscopy systems to provide tissue diagnosis in real-time, noninvasively, and objectively (with the use of multivariate data analysis). [12]

Techniques such as fluo rescence spectroscopy, reflectance spectroscopy, elastic scattering spectroscopy, infrared spectroscopy, and Raman spectroscopy (RS) are increasingly being investigated for oral cancer applications. Raman spectroscopy RS is a vibrational spectroscopy method based on the inelastic scattering of light. Inelastic scattering of light, also known as Raman effect, was discovered by Sir C. V. Raman after seminal experiments on scattering.

This effect was discovered in the year 1928, for which Raman received the Nobel Prize in 1930. When a sample is irradiated with intense monochromatic light, phenomena such as absorption, scattering, and reflection occur. Most of the scattered photons have the same frequency of the incident light(Rayleigh scattering) while a small proportion (one in ten million) are inelastically scattered, i.e. with a frequency different from the incident photons; this phenomenon is termed as Raman effect. When the frequency of the scattered light is lower than the frequency of incident photon, the process is called Stokes shift. If the frequency of scattered photon is higher than incident photon, the process is called anti-stokes shift. [13]

Instrumentation

RS is an inherently weak process: Only 1 in 10 million photons are Raman scattered. Thus, sophisticated instrumentation, i.e. powerful excitation source, high-

throughput spectrograph, and sensitive detection systems are a prerequisite. The introduction of low-noise charged coupled device (CCD) detector technology, highly efficient imaging spectrographs, and compact semiconductor laser excitation sources enabled extensive Raman spectroscopic applications in diverse areas.

Typically, Raman spectrometer is made up of

(i) Excitation source,

(ii) Optical system,

(iii) Spectrograph, and

(iv) Detection and computer control/processing systems.

For most applications, a continuous-wave laser is employed as the excitation source in Raman spectrometers. The optical system consists of light steering mechanisms which direct and select laser wavelength for sample excitation.[14]

Rayleigh rejection system, which prevents elastically scattered light to be incident on the spectrograph and detector, is the most crucial component of Raman system as it facilitates the rejection of Rayleigh scattered light and detection of the comparatively weaker Raman scattered light. The main function of the spectrograph is to disperse light into its component wavelengths. CCDs are the most commonly employed detectors for RS in recent times.[15]

Application

Due to attributes such as sensitivity, high information content, and nondestructive nature, RS has been extensively applied in the fields of chemistry, biology, geology, pharmacology, forensics, pharmaceuticals, and material sciences. It is known that disease is accompanied with a concomitant change in native tissue biochemistry.[16]

IRS can detect these changes and facilitate disease diagnosis. This is the basis for Raman spectroscopic diagnosis of diseases, including cancers. IRS has shown that potential in diagnosis of several diseases including cancers, both ex vivo and in vivo. IRS has extensively been employed for diagnosis of oral cancers.

Oral cancer applications

The first Raman spectroscopic applications in oral cancers were investigated by Bakker Schut et al. in 2000. This group explored the in vivo classification of normal and dysplastic tissue in rat palate after cancer was induced by application of 4-nitroquinoline 1-oxide. Since then, several studies have investigated the potential of RS in the management of oral cancers. Different approaches - ex vivo, in vivo, biofluids, cell based, and imaging have been explored.[17]

Diagnosis

Ex vivo studies

The animal study by Bakker Schut et al. was followed by a study on human oral frozen cancer biopsies by Venkatakrishna et al. in 2001. Raman spectroscopic measurements from formalin-fixed tissues were consequently demonstrated, and significant difference between the normal and malignant epithelial regions was observed. Malini et al. in the year 2006, carried out an extended study to discriminate normal, cancerous, precancerous, and inflammatory conditions. Lipid-rich features and predominant protein features were observed in normal and tumor conditions, respectively. Classification between different groups was explored using PCA coupled with multiparametric “limit test”, and high sensitivity and specificity were achieved.

Hu et al. acquired spectra of 66 human oral mucosa tissues (43 normal and 23 malignant) using confocal Raman microspectroscopy in 2008. After preprocessing spectra using wavelet-based analysis, PCA along with the calculation of areas under bands 1004, 1156, 1360, 1587, and 1660/cm was used as a classification method.[18]

Findings demonstrated that oral carcinomas of different pathological grades can also be identified with RS. Shifted-excitation Raman difference spectroscopy study (SERDS) on 12 oral squamous cell carcinoma (OSCC) tissues could differentiate between malignant and benign areas with sensitivity of 86% and specificity of 94%. Keratin as a marker for OSCC identification using RS was recently demonstrated on 24 tissues samples with a sensitivity and specificity of 77–92% and 100%, respectively.[19]

Rapid detection of oral cancer using 24 normal and 32 oral tumor sections on Ag-TiO2 nanostructured SERS substrate has been recently shown, achieving 100% sensitivity and 95.83% specificity.Following these successful ex vivo studies on both fixed and frozen tissues, in vivo oral cancer studies on humans were concomitantly initiated.[20]

In vivo studies

The first in vivo Raman spectroscopic study on humans was carried out by Guze et al.for identifying site wise variations in the human oral cavity. In this study, the feasibility of spectral acquisition from oral cavity, reproducibility of Raman spectroscopic signature of normal oral mucosa among different anatomical oral sites was evaluated on 51 subjects of different races (Asian and Caucasian) and genders. [21]

This study, carried out on high-wavenumber region, suggested that spectra for different oral sites within the same ethnic group are significantly different, and the Raman signal was not influenced by gender or ethnicity. The differences between anatomical subsites could be due to varying degrees of keratinization.[22]

Raman Imaging

Raman mapping experiments on oral mucosal tissues have also been reported. As oral mucosa is not homogenous and comprises different layers and histological characteristics, signal contributions from individual layers have to be understood. In the first study by Cals et al.,[45] the method of RS-based histopathology was developed and standardized. The study revolved around Raman microspectroscopic mapping of unstained frozen sections, followed by histopathological annotation of features in Raman images. Twenty experiments were conducted on different tissue sections obtained from tongue SCC; K-means cluster analysis(KCA) and HCA were used for data analysis. Findings indicated Raman mapping followed by KCA and HCA, can be used as a reproducible method to effectively define the spectral characteristics of individual histopathological structures for oral mucosa. In a subsequent study by Daniel et al.[23,24]

Raman mapping was explored for oral cancer diagnosis. Normal and oral cancer tissue sections could be distinguished based on the spectral parameters. PCA and KCA were employed to construct pseudocolor images. Raman maps could clearly delineate tumor margins. Mapping carried out on a blind sample also yielded correct identification of the sample. A similar study by our group[aimed to understand biochemical variations in normal and malignant oral buccal mucosa. Data were acquired from 10 normal and SCC tissues. Raman maps of normal sections could resolve the layers of epithelium, i.e. basal, intermediate, and superficial while inflammatory, tumor, and stromal regions were identified in tumor maps. PCA could successfully classify epithelium and stromal regions of normal cells. The classification between cellular components of normal and tumor sections was also observed.[25,26]

Surgical Margin Assessment

Tumor-positive resection margins lead to recurrence in oral cancer patients and consequently lower disease-free

survival rates. The sensitivity of RS could be exploited for the detection of surgical margins in oral cancer tissues. Potential of RS in surgical demarcation was investigated by two recent studies. In the first study by Barroso et al. differential water content in malignant and surrounding normal tissue was used as a basis for identifying surgical margins using Raman bands of OH- and CH-stretching vibrations in high-wavenumber region.[27,28]

The water content in SCC was significantly higher than surrounding healthy tissue. Thus, tumor tissue could be detected with a sensitivity of 99% and a specificity of 92% after using a cutoff water content value of 69%. In another study by Cals et al.Raman imaging of normal and tissue sections from ten oral cancer patients was carried out and 127 pseudo-color Raman images were generated. These images were linked to the histopathological evaluation of same sections, and spectra were annotated based on histopathological findings. [29]

Thus, RS could successfully differentiate tumor and surrounding healthy tissues. As Raman measurements are fast and can be carried out on freshly excised tissue without any preparation, the development of an intraoperative tool for guiding tumor resection may improve patient outcomes.[30]

Conclusion

Oral cancers are associated with poor disease-free survival rates. Improvements in screening, diagnostic, and monitoring approaches can lead to improved treatment outcomes. Raman spectroscopic applications in oral cancer have been extensively investigated. These studies have demonstrated the potential of RS in being an objective; real-time screening, diagnostic, and therapeutic monitoring adjunct for oral cancer diagnosis. Raman imaging studies have helped in understanding the spectral contributions from the different layers of the epithelium; further studies on rapid scanning methods can help in real-time surgical demarcation. The studies on margin assessment have shown feasibility of clearly differentiating tumor from surrounding normal using high-wavenumber region and Raman imaging. The studies on prediction of treatment response have successfully identified recurrence-prone patients and changes associated with the acquisition of radio-resistance in a cell-line model.

Overall, these studies have strongly demonstrated the potential of RS and preparedness of this instrument for noninvasive and less-invasive diagnosis of oral cancers. Translation of this approach to clinics may help in improved preliminary oral cancer screening, early diagnosis, and enhance disease-free survival rates.

References

1. Kirita T, Omura K. Oral Cancer: Diagnosis and Therapy. Tokyo: Springer; 2015.
2. Carnelio S, Rodrigues G. Oral cancer at a glance. Internet J Dent Sci 2004;1:1-12.
3. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. Oral Oncol 2009;45:309-16.
4. Coelho KR. Challenges of the oral cancer burden in India. J Cancer Epidemiol 2012;2012:701932.
5. Khandekar S, Bagdey P, Tiwari R. Oral cancer and some epidemiological factors: A hospital based study. Indian J Community Med 2006;31:157-9.
6. Mehrotra R, Gupta DK. Exciting new advances in oral cancer diagnosis: Avenues to early detection. Head Neck Oncol 2011;3:33.
7. Messadi DV. Diagnostic aids for detection of oral precancerous conditions. Int J Oral Sci 2013;5:59-65.

8. Swinson B, Jerjes W, El-Maaytah M, Norris P, Hopper C. Optical techniques in diagnosis of head and neck malignancy. Oral Oncol 2006;42:221-8.

9. Raman CV, Krishnan KS. A new type of secondary radiation. Nature 1928;121:501-2.

10. Haynes CL, McFarland AD, Duyne RPV. Surface-enhanced Raman spectroscopy. Anal Chem 2005 ; 77 : 338 A - 46A.

11. Zhang D, Xie Y, Mrozek MF, Ortiz C, Davisson VJ, Ben-Amotz D. Raman detection of proteomic analytes. Anal Chem 2003;75:5703-9.

12. Begley R, Harvey A, Byer RL. Coherent anti-stokes Raman spectroscopy. Appl Phys Lett 1974;25:387-90.

13. Bakker Schut TC, Witjes MJ, Sterenborg HJ, Speelman OC, Roodenburg JL, Marple ET, et al. In vivo detection of dysplastic tissue by Raman spectroscopy. Anal Chem 2000;72:6010-8.

14. Venkatakrishna K, Kurien J, Pai KM, Valiathan M, Kumar NN, Murali Krishna C, et al. Optical pathology of oral tissue: A Raman spectroscopy diagnostic method. Curr Sci 2001;80:665-9.

15. Krishna CM, Sockalingum GD, Kurien J, Rao L, Venteo L, Pluot M, et al. Micro-Raman spectroscopy for optical pathology of oral squamous cell carcinoma. Appl Spectrosc 2004;58:1128-35.

16. Malini R, Venkatakrishna K, Kurien J, Pai KM, Rao L, Kartha VB, et al. Discrimination of normal, inflammatory, premalignant, and malignant oral tissue: A Raman spectroscopy study. Biopolymers 2006;81:179-93.

17. Hu Y, Jiang T, Zhao Z, editors. Discrimination of Squamous Cell Carcinoma of the Oral Cavity Using Raman Spectroscopy and Chemometric Analysis. Intelligent Networks and Intelligent Systems, 2008 ICINIS'08 First International Conference on; 2008, IEEE.

18. Sunder N, Rao N, Kartha V, Ullas G, Kurien J. Laser raman spectroscopy: A novel diagnostic tool for oral cancer. J Orofac Sci 2011;3:15.

19. Christian K, Johanna M, Werner A, Kathrin B, Tesfay GM, Robert H, et al. Raman difference spectroscopy: A non-invasive method for identification of oral squamous cell carcinoma. Biomed Opt Express 2014;5:3252-65.

20. Chen PH, Shimada R, Yabumoto S, Okajima H, Ando M, Chang CT, et al. Automatic and objective oral cancer diagnosis by Raman spectroscopic detection of keratin with multivariate curve resolution analysis. Sci Rep 2016;6:20097.

21. Girish CM, Iyer S, Thankappan K, Rani VD, Gowd GS, Menon D, et al. Rapid detection of oral cancer using Ag-TiO 2 nanostructured surface-enhanced Raman spectroscopic substrates. J Mater Chem B 2014;2:989-98.

22. Guze K, Short M, Sonis S, Karimbux N, Chan J, Zeng H. Parameters defining the potential applicability of Raman spectroscopy as a diagnostic tool for oral disease. J Biomed Opt 2009;14:014016.

23. Bergholt MS, Zheng W, Lin K, Ho KY, Teh M, Yeoh KG, et al. Characterizing variability in in vivo Raman spectra of different anatomical locations in the upper gastrointestinal tract toward cancer detection. J Biomed Opt 2011;16:037003.

24. Krishna H, Majumder SK, Chaturvedi P, Gupta PK. Anatomical variability of in vivo Raman spectra of normal oral cavity and its effect on oral tissue classification. Biomed Spectrosc Imaging 2013;2:199-217.

25. Singh SP, Deshmukh A, Chaturvedi P, Krishna CM. Raman spectroscopy in head and neck cancers: Toward oncological applications. J Cancer Res Ther 2012;8 Suppl 1:S126-32.

26. Singh S, Deshmukh A, Chaturvedi P, Krishna CM, editors. In Vivo Raman Spectroscopy for Oral Cancers Diagnosis. Bellingham, Washington, USA: SPIE BiOS, International Society for Optics and Photonics; 2012.

27. Singh SP, Deshmukh A, Chaturvedi P, Murali Krishna C. In vivo Raman spectroscopic identification of premalignant lesions in oral buccal mucosa. J Biomed Opt 2012;17:105002.

28. Deshmukh A, Singh SP, Chaturvedi P, Krishna CM. Raman spectroscopy of normal oral buccal mucosa tissues: Study on intact and incised biopsies. J Biomed Opt 2011;16:127004.

29. Sahu A, Deshmukh A, Ghanate AD, Singh SP, Chaturvedi P, Krishna CM. Raman spectroscopy of oral buccal mucosa: A study on age-related physiological changes and tobacco-related pathological changes. Technol Cancer Res Treat 2012;11:529-41.

30. Singh SP, Sahu A, Deshmukh A, Chaturvedi P, Krishna CM. In vivo Raman spectroscopy of oral buccal mucosa: A study on malignancy associated changes (MAC)/cancer field effects (CFE). Analyst 2013;138:4175-82.