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Infra red spectroscopy in oral cancer- An update on Fourier transform infrared spectroscopic applications

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Abstract

Oral cancers are known to suffer from dismal survival rates, which have not improved for several decades. As delayed diagnosis contributes to the low disease-free survival rate observed in oral cancers, RS has also been explored for the early diagnosis of oral cancers.

Raman spectroscopy (RS) is a sensitive vibrational spectroscopic method that can detect even subtle biochemical changes during the onset of disease. Consequently, RS has been extensively investigated for disease diagnosis, including cancers.

Use of spectroscopy for detection of cancer is more reliable as compared to any other techniques. We have implemented the FTIR spectroscopic techniques for the detection of 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. We have obtained FTIR spectra of malignant and non-malignant histological samples of oral tissues and differentiate the spectra using origin software.

This review also summarizes the major developments in the field, including diagnosis, surgical margin assessment and prediction of treatment response, and in the overall management of oral cancers.

The present article comprises an overview of epidemiology, diagnosis, treatment, and recently introduced diagnostic adjuncts for oral pre-cancerous lesions and oral cancer, the basic principle, instrumentation of FTIR, multivariate analysis that impart objectivity to the approach, and finally a discussion on the recent applications in oral cancers.

Keywords: Precancerous lesions, Raman spectral analysis, Oral neoplasms.

Introduction

Oral cancer is the eighth most common cancer worldwide with an estimated 657,000 new cases and 330,000 deaths annually in 2020, and these numbers are expected to double by 2035 according to the World Health Organization (WHO)[1].

Despite easy access to the oral cavity for examination and significant advances in treatment, oral cancer

patients often face very high morbidity and mortality rates due to late-stage diagnosis, which accounts for approximately 70% of all new cases[2].

The 5-year survival rate for oral cancer patients ranges from 20 to 90% depending on the stage of diagnosis[3]. Early-stage oral cancer often manifests as subtle mucosal lesions classified as oral potentially malignant disorders (OPMD)[4]. Early detection and effective management of these lesions are critical for improving survival rates and preventing oral cancer progression[5].

The gold standard for oral cancer diagnosis is biopsy and subsequent histopathological evaluation under a microscope. This process is invasive, time-consuming, and subject to inter-observer variability[6].

Furthermore, histopathological assessment based on tissue morphological alterations does not provide an accurate risk assessment for OPMDs and tends to detect oral cancer at late stages[7].

Various adjunctive techniques have been proposed to facilitate the screening and diagnosis of oral cancer such as exfoliative cytology (cyto brush)[8], vital tissue staining[9], and the use of chemo luminescence or autofluorescence[10,11,12]. However, despite the continuous effort of improvement, most techniques still exhibit limited ability to provide accurate information and help clinicians detect oral cancer in early stage [13].

Recently, molecular markers and salivary tests have been investigated for their potential in early oral cancer detection[14,15,16]. However, so far, no single biomarker can reliably validate the presence or predict the prognosis of oral cancer[17,18].

The search for a fast, simple, accurate, and cost-effective diagnostic method for early oral cancer detection is still underway. One promising technique is Fourier transform infrared (FTIR) spectroscopy, which provides molecular fingerprints of biological samples based on vibrational transitions of chemical bonds in the samples upon interaction with infrared light. FTIR is a non-invasive and label free method that can detect early bimolecular changes associated with a neoplasm condition even before the emergence of morphological abnormalities, which strongly supports its role in early cancer detection[19,20].

To date, considerable research work has demonstrated the competitive to superior performance of FTIR in comparison to conventional cancer screening and diagnostic techniques, making it a potentially powerful clinical tool in modern medicine[21,22,23,24].

Hence this review is aimed at focusing the importance of infra-red spectroscopy in oral precancerous and cancerous lesions.

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. The energy difference between the incident and scattered photon (Raman shift) is represented as wavenumber (/cm)

Common oral pre-cancerous lesions

It is well established that oral SCC occurs as a result of several molecular and biochemical cellular alterations and changes in the underlying fibrovascular stroma including neovascularisation. In conjunction with cellular alterations, clinical changes in the affected epithelial tissues are observed as well, known as precancerous lesions. The clinical significance of oral precancerous lesions lies in its association with malignant transformation into OSCC[25,26]

The most common precancerous lesions present clinically as white, red or a mix of white and red mucosal changes. These clinical conditions are known as leukoplakia or erythroplakia. There are other pathological conditions that are considered precancerous including oral lichen planus and oral submucous fibrosis. In addition, less common lesions include discoid lupus erythematosus and some rare hereditary conditions such as dyskeraotisis congenita and epidermolysis bullosa[26].

The malignant potential of the above-mentioned oral lesions cannot be accurately predicted solely on the basis of their clinical characteristics, histological evaluation is essential for all suspicious lesions.

Unfortunately, histological findings only indicate that a given lesion may have malignant potential (dysplasia), and cannot be used for the prediction of malignant changes. Thus, the presence of dysplasia only indicates that an oral lesion may have an increased risk of malignant transformation. Molecular biomarkers capable of identifying the subset of lesions likely to progress to cancer are being widely investigated including genetic and epigenetic alterations observed in oral mucosal precancerous lesions[26]

Fourier transform infrared spectroscopy/micro spectroscopy

FTIR spectroscopy is an established analytical technique with diverse applications. It was traditionally used by chemists to characterize the molecular structures of a material. Molecules have discrete energy levels for electronic transitions, molecular vibrations, and molecular rotations.

When a molecule is irradiated by infrared light, it absorbs a certain amount of the incident radiation at a specific energy/frequency and undergoes vibrational excitation from the ground state to a higher vibrational energy state. The unique pattern of infrared absorption by a particular molecule or functional group produces characteristic bands in their FTIR spectra.

The band position is affected by the mass of vibration, the type of molecular bond (e.g., single or double bond), the intra- and inter-molecular environment, and the coupling with other vibrations; the band height is proportional to the concentration of corresponding chemical moieties; and the band width provides an estimate of intermolecular interactions. FTIR spectroscopy provides a biochemical profile of proteins, nucleic acids, lipids, and carbohydrates in a biological sample, called “biomolecular fingerprinting” [27,28]and is sensitive enough to probe subtle changes in the molecular structure and microenvironment such as the secondary structure of proteins, the mutation of nucleic acids, and the peroxidation of phospholipids [29,30,31]

Ftir and oral pre malignant lesions- A target for early oral cancer detection

The earliest detectable morphologic changes of oral cancer are the appearance of the ‘precancerous’ lesions,

of which the most common ones are leukoplakia and erythroplakia. Oral leukoplakia, a white lesion in the mucosa of the oral cavity, represents the most common precursor lesion of OSCC and its prevalence varies between 0.1% and 0.5%.

Unfortunately, the reported proportion of oral leukoplakia that develops into oral cancer varies depending on several factors including, the study population, the definition of leukoplakia used and the length of observation time, but an annual transformation rate of 1%–2% per year is a reasonable assumption.

Imaging using FTIR microscope allows analysis of biochemical compounds and is a technique well suited for individual cells and tissue analysis. It provides information about the biochemical nature of tissue or cell samples and has been applied in many different areas of medical research and testing. IR absorption spectra of abnormal tissues and normal tissues are compared by lipid (2800 - 3000 cm−1), protein (1500 - 1700 cm−1), and nucleic acids (1000 - 1250 cm−1 regions. OLK is one of the mucous membrane lesions of the mouth is. This change has a “fingerprint region” in the range of 900 - 1800 cm−1

This metabolomics study can be used in the study of the molecular properties of the abnormal lipoproteins from the plasma and is considered to be a great step in the blood screening for an early detection of OSCC. This kind of the technique is expected to provide a useful tool in the diagnosis of oral cancer in the future.

Ftir spectroscopy and 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[32].

In vivo studies

The first in vivo FTIR 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 FTIR spectroscopic signature of normal oral mucosa among different anatomical oral sites was evaluated on 51 subjects of different races (Asian and Caucasian) and genders.

This study, carried out on high-wave number region, suggested that spectra for different oral sites within the same ethnic group are significantly different, and the IR signal was not influenced by gender or ethnicity.

The differences between anatomical subsites could be due to varying degrees of keratinization. Therefore, the study suggested clustering of sites based on anatomical and spectral similarities. The consequent study by Bergholt et al aimed to characterize the in vivo Raman spectroscopic properties of different normal oral tissues in the fingerprint region (800–1800/cm) and to understand the biochemical basis for differentiation[33].

In another study, the origin of IR signals in tissues was also investigated. The effect of age-related physiological changes and their possible influence in classification between normal and pathological conditions was also explored by our group[34].Next, the feasibility of detection of malignancy-associated-changes,orcancer-field-effects (CFEs) in oral cancer patients was evaluated. Spectra were acquired from 84 subjects and subjected to PC-LDA. Findings indicate differences between contralateral normal sites of tobacco and nontobacco habitués. Contralateral normal of nonhabitue oral cancer subjects was distinct from healthy controls, suggesting

microarchitectural changes that characterize CFE could be detected using in vivo RS[35].

All these studies explored the detection of cancer and precancer lesions on buccal mucosa subsite. Krishna et al. investigated the classification between spectra acquired from multiple normal sites and histopathologic ally verified precancer (OSMF and leukoplakia) and cancer lesions. Using probability-based multiclass diagnostic algorithm, sensitivity and specificity of 94.2% and 94.4% were obtained for a binary normal versus abnormal model.

In a recent study by Guze et al**.,** IR spectral discrimination of premalignant and malignant lesions from contralateral normal and benign lesions was explored. Spectral differences were apparent between groups; the premalignant and malignant lesions could be classified with 100% sensitivity and 77% specificity.

Further, the study also states that the anatomic origin of the tumour may not have a bearing on normal versus tumour classification. Another recent study by our group has shown major spectral differences between buccal mucosa and tongue while the lip was shown to have an intermediate position. Individual and pooled subsite diagnostic algorithms were explored with respect to spectra and classification[36].

Ftir imaging

FTIR 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., the method of FTIR-based histopathology was developed and standardized[37].

The study revolved around IR micro spectroscopic 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 FTIR 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 pseudo color images. 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[38].

FTIR maps of normal sections could resolve the layers of epithelium, i.e., basal, intermediate, and superficial while inflammatory, tumour, and stromal regions were identified in tumour maps. PCA could successfully classify epithelium and stromal regions of normal cells. The classification between cellular components of normal and tumour sections was also observed

Surgical margin assessment

Tumor-positive resection margins lead to recurrence in oral cancer patients and consequently lower disease-free survival rates. The sensitivity of FTIR could be exploited for the detection of surgical margins in oral cancer tissues. Potential of FTIR 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.

The water content in SCC was significantly higher than surrounding healthy tissue. Thus, tumour 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 pseudos-color Raman images were generated.

These images were linked to the histopathological evaluation of same sections, and spectra were annotated based on histopathological findings. Thus, FTIR could successfully differentiate tumour 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 tumour resection may improve patient outcomes[39]

Prediction of treatment response

FTIR spectra have been shown to correlate with molecular and cellular changes associated with disease, including cancer. RS can therefore be used for monitoring treatment response in oral cancers. In a recent retrospective study by our group, serum RS could differentiate between cohorts of patients with or without recurrence. DNA and protein content were major spectral features to differentiate the recurrence and nonrecurrence groups [40].

Prediction of radio response by RS was investigated in vitro using radio resistant oral cancer cell lines established by fractionated radiation[41].

FTIR spectral differences were observed between parental tongue cancer cell line, 50 Gy, and 70 Gy cell lines; PCA also yielded three distinct clusters. The change in molecular profile acquired by radio resistant sublines was successfully detected by FTIR.

Conclusions

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 FTIR in being an objective; real-time screening, diagnostic, and therapeutic monitoring adjunct for oral cancer diagnosis. In vivo approaches have demonstrated the potential of FTIR in screening and early diagnosis of abnormal mucosal conditions at all oral subsites.

The studies on minimally invasive samples, including serum, have shown potential in preliminary screening of oral cancers; urine-based approaches have shown promising results which need to be validated on a large sample size. FTIR of exfoliated cells has shown promise in differentiating normal and tumour samples and in identifying early precancerous changes in cell lines. 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 tumour from surrounding normal using high-wave number region and FTIR 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 FTIR and preparedness of this instrument for

non-invasive 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.

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