Spectroscopy: A New Diagnostic Technique for Detection of Potentially Malignant Oral Lesions

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ABSTRACT

The common procedure for detecting potentially malignant (PMOL) or malignant oral lesions consists of visual inspection, followed by biopsy of any suspicious lesions found. However, the processing of biopsy material and the interpretation of the results inevitably leads to diagnostic delay and the added possibility of taking an unrepresentative sample. Therefore, techniques that can distinguish between benign from malignant types in a reliable and noninvasive way would be very useful. This review provides an overview of the literature how spectroscopic techniques are useful and can provide tissue diagnosis in real-time, noninvasively and in situ.

Key message: Spectroscopic techniques can provide tissue diagnosis in real-time, non-invasively and in situ.

Keywords: Spectroscopy, Optical techniques, Early detection, Potentially malignant oral lesions.

INTRODUCTION

A systematic review of the scientific literature was done in preparation of this manuscript. Database of indexed journals (PubMed, Sciencedirect and Ovid) and search engines like Google was searched. Database of indexed journals was searched for keywords like spectroscopy, optical techniques, early detection, oral cancer and potentially malignant oral lesions.

Oral cancer is known to develop from pre-existing potentially malignant oral lesions (PMOL) or de novo. They are first diagnosed when they become symptomatic. By this stage approximately two third of patients would have already developed advanced disease with regional metastasis and have a consequently diminished prognosis. The subsequent treatment would require surgery and adjunct radiotherapy, with its attendant high rate of morbidity and mortality. If they are diagnosed and treated at an early stage, then both survival rate and quality of life can be improved. Early diagnosis is therefore of paramount importance.

The common procedure for detecting PMOL consists of visual inspection, followed by biopsy of any suspicious lesions found. However, benign lesions which are very common and diverse, may present themselves very similar to early malignant or PMOL's, which makes difficult to distinguish, even for experienced clinicians.

Therefore, technique or a device that can distinguish between different lesion types, its malignant potential, appropriate biopsy site, planning treatment, its outcome, prognosis in a reliable and noninvasive way would be very useful. Many techniques to date have been reviewed so far, e.g. vital staining procedure (Toluidine blue and Lugol’s iodine), Brush biopsy (Oral CDx Brush), light based detection system, Micronuclei analysis, DNA Ploidy, etc. but have certain limitations.

Recently, there has been increased interest in optical systems using tissue spectroscopy to establish diagnosis. Spectroscopy was originally the study of interaction between radiation and matter as a function of wavelength. In fact, historically, spectroscopy refers to the use of visible light dispersed according to its wavelength, e.g. by a prism. Spectrometry is the spectroscopic technique used to assess the concentration or amount of a given species. Instrument that performs such measurements is called as spectrometer or spectrograph. Spectroscopy/spectrometry is often used in physical and analytical chemistry for the identification of substances through the spectrum emitted from or absorbed by them.

Optical spectroscopy explores the optical phenomena resulting from the interaction of light with biological tissue. It may be particularly useful for the analysis of differences in-between normal and cancerous tissue because of major scattering, absorption and fluorescence changes which are known to occur during the development of cancer. Optical spectroscopy has the potential to detect malignant lesions earlier, before they become macroscopically visible, by probing tissue biochemistry and morphology in vivo in real time.

Three optical techniques that are currently utilized in the detection of PMOL and oral malignancies are; Fluorescence, Elastic scattering and Raman spectroscopy.

FLUORESCENCE SPECTROSCOPY (FS)

Fluorescence spectroscopy also called as fluorometry or spectrofluorometry. It is a type of electromagnetic spectroscopy which analyzes fluorescence from a sample. It involves using a
beam of light, usually ultraviolet light, that excites the electrons in molecules of certain compounds and causes them to emit light of a lower energy typically, but not necessarily visible light. Devices that measure fluorescence are called fluorometers or fluorimeters.

This technique was first described by Alfano et al. they use autofluorescence spectroscopy in vivo, to differentiate between normal and malignant tissues. This technique has also recently been used to map out the individual characteristics of healthy oral mucosa at several anatomical sites within the oral cavity and is used as a baseline for further studies.

**Principles of Fluorescence Spectroscopy**

When cells interact with light they become excited and re-emit light of varying colors (fluorescence). This can be detected by sensitive spectrometers. All tissues fluoresce due to the presence of fluorescent chromophores (fluorophores) within them. Characteristic spectra reflect biochemical changes occurring within the tissue. The resultant spectra not only detect the light that is fluoresced but also are sensitive to the structures that absorb light, e.g. hemoglobin.

The commonly detected fluorophores are NADH, collagen, elastin and co-factors such as flavins (FAD, FMN). The fluorescence can either occur as autofluorescence (if induced by UV light), or as a laser induced phenomenon and may also be enhanced by either topical or systemic application of 5-aminolevulinic acid (ALA).

Dysplastic and malignant tissues have different spectral characteristics. They tend to show increased red fluorescence and decreased green fluorescence. Significant increase in the red/green fluorescence ratio is an accurate predictor of dysplasia and malignancy. Malignant tissue also has a limited ability to metabolize iron, so that an exogenous application of ALA will result in an intracellular increase in protoporphyrin IX which increases tissue fluorescence.

Gillenwater et al (1998) used autofluorescence to look at neoplastic and non-neoplastic oral mucosa and found that the fluorescence intensities were less for abnormal than normal sites. They showed that the ratio of red spectrum (> 600 nm) to the blue spectrum (455-490 nm) was greater in areas of abnormal disease. By using peak intensity at 337 nm, they were also able to obtain a sensitivity of 88% and a specificity of 100%.

Onizawa et al (1999) used fluorescence spectroscopy for diagnosing oral cancer. 130 oral lesions from 130 patients were subjected to fluorescence spectroscopy. 72/79 (91.1%) of carcinoma and 6/7 (85.7%) of epithelial dysplasia were identified. They suggest that fluorescence spectroscopy is a useful tool in oral tissue pathology diagnosis.

Van Staveren HJ et al (2000) used autofluorescence to distinguish oral dysplasia from normal mucosa using an artificial neural network and revealed a sensitivity and specificity of 86% and 100% respectively.

Sharwani A et al (2006) used fluorescence spectroscopy in combination with 5-aminolevulinic acid-induced protoporphyrin IX fluorescence in detecting oral premalignancy. They concluded that the technique was found as a valuable tool in the diagnosis of oral premalignancy. This technique offers the potential to be advantageous over other nonoptical techniques in terms of providing real-time diagnosis, in situ monitoring, cost effectiveness and more tolerated by patient compared to surgical biopsy.

**ELASTIC SCATTERING SPECTROSCOPY (ESS)**

ESS has the advantage of being fast, reliable and cost-effective and potentially offers a diagnosis in situ, non-invasively and in real time.

**Principles of Elastic Scattering Spectroscopy**

The principle behind this technique is generation of wavelength dependant spectrum that reflects structural and morphological change within tissues. Elastic scattering implies that the light returns with the same kinetic energy as the incident photons. The incident light can undergo single, or more commonly, multiple scattering events before being collected again at the same surface by an optical probe and the data is analyzed. The acquired data reflects both the scattering and absorptive properties of that tissue. This scattering process has been shown to occur at gradients in the optical index of refraction resulting from differences in densities that occur at a cellular and subcellular level. The structures that induce the scattering (scattering centers) are the nucleus, chromatin concentration, and subcellular organelles.

Muller et al (2003) used ESS to look at normal versus abnormal tissue and dysplastic versus cancerous in the oral cavity. When comparing spectroscopy to histopathology his accuracy for normal was 91.6%, abnormal (97%), dysplasia 64.3% and carcinoma 50%.

Thus, ESS has been shown to be sensitive to nuclear size, chromatin content, nuclear/cytoplasmic ratio and cellular crowding, which are criteria that the histopathologist looks for when establishing of malignancy within a tissue.

**RAMAN SPECTROSCOPY (RS)**

Although the inelastic scattering of light was predicted by Smeak in 1923, it was not until 1928 that it was observed in practice. The Raman effect was named after the Indian scientist Sir CV Raman who observed the effect by means of sunlight (1928, together with KS Krishnan and independently by Grigory Landsberg and Leonid Mandelstam). Sir Raman won the Nobel Prize in Physics in 1930 for this discovery.

**Principles of Raman Spectroscopy**

A Raman spectrum is a form of in-elastic scattering and is generated by shift in frequency in the incident excitation light. This is caused by discrete changes in emergent light, above and below the wavelength of the incident photons due to the vibrational frequencies of the biomolecules that constitute the tissue. This technique is extremely sensitive and is the most accurate of the techniques but the signal is extremely weak, in the order of one trillionth of the incident beam.
Within biological tissues there are four principle components that contribute to the spectra; water, lipids (cell membranes), nucleic acids (DNA and RNA) and proteins (hormones, isoenzymes, immunoglobulins and keratins). The resultant spectra from these structures give a characteristic signature for that tissue. Operator can change wavelength and hence can probe different depths of tissue due to different wavelength penetrations and the technique therefore represents a true form of optical histochemistry.\(^\text{14}\)

Raman spectroscopy has rarely been used in isolation in the investigation of oral malignancy but is utilized in conjunction with ESS and fluorescence. The major disadvantage of this technique is that, it is expensive and complex.\(^\text{5}\)

Stone N et al (2000) used Raman spectroscopy to differentiate premalignant and malignant lesions from normal laryngeal mucosa. They concluded that there was a strong evidence to support spectral identification of malignancy and earlier abnormal changes.\(^\text{15}\)

Lau et al (2003) investigated the reliability of Raman spectroscopy in differentiating between normal and cancerous tissue. They suggested that Raman spectroscopy could be a useful tool to distinguish cancer from normal tissue.\(^\text{16}\)

Malini R et al (2006) used Raman spectroscopy for discrimination of normal, inflammatory, premalignant, and malignant oral tissue. They concluded that the biochemical differences between normal and pathological conditions of oral tissue showed different classes of spectra, hence discriminating the lesions.\(^\text{17}\)

**TRIOMODAL SPECTROSCOPY (TS)**

The accuracy of the techniques can be increased by combining all three methods and this is known as Trimodal spectroscopy. This is an expensive and time consuming technique.\(^\text{2}\)

Muller et al (2003) used ESS to look at normal vs abnormal tissue and dysplastic vs cancers in the oral cavity. When comparing spectroscopy to histopathology his accuracy for normal was 91.6% (22/24) and 97% (33/34) for abnormal. The figures fell when examining dysplasia 64.3% (9/14) and carcinoma 50% (5/10). However, when using Trimodal spectroscopy it showed a sensitivity and specificity of 96% when comparing cancerous/dysplastic from normal and figures of 64% and 90%, respectively, when comparing dysplastic from cancerous.\(^\text{5}\)

**CONCLUSION**

This paper reviews various spectroscopic techniques (optical techniques) used in detection of potentially malignant and malignant oral lesions. Understanding the development and progression of these lesions is a key in the quest for the early diagnosis and prevention. The rich source of information and diagnostic potential provided by spectroscopic techniques allows us to understand more fully the changes that take place during the onset and progression. These techniques not only help in differentiating between normal mucosa from dysplastic or malignant lesions but also can help in monitoring treatment and potential complications.

These techniques can provide diagnosis in real-time and are non-invasive. At present, large multicentric trials are necessary to determine the sensitivity and specificity of these individual and combined techniques, to assess and to improve their ability in detection and management of potentially malignant and malignant oral lesions.

**REFERENCES**