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Research Article

AUTOFLUORESCENCE SPECTROSCOPY: AN EMERGING TOOL FOR ORAL DIAGNOSIS

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ABSTRACT

Oral cancer is among the ten most common cancer worldwide and is a significant health problem throughout the world. Early detection and prompt treatment offers the best chance for cure. As patient's awareness regarding the danger of oral cancer increases the demand for screening is expected to increase. Any tool that improves the early detection of lesions can effectively improvise oral cancer screening system. Many light based noninvasive diagnostic techniques have been developed that aid in the early detection of oral cancer. Among these autofluorescence spectroscopy is emerging as one of the most potential and dynamic tool in the early diagnosis of oral cancer. Here we highlight the importance of the science of autofluorescence spectroscopy in the early detection of premalignant and in situ malignant lesions and the role of stomatologists.

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INTRODUCTION

The field of optical diagnostics comprises variety of techniques designed to characterize the relationship between the optical and biological properties of tissue.[1] Through the detection of changes in light after intervention with tissue, optical technologies provide information on the physiologic and pathologic correlation of the tissue at a molecular level. Early research in optical diagnostics suggested that alterations in light-tissue interactions can be used to differentiate normal from malignant tissue.[2] Subsequent advances in molecular biology, genomics, and proteomics, have vastly improved our scientific understanding of the complex biochemical and morphological changes that occur as tissue undergoes the transformation from normal to neoplasia. Many of these early biological events have been shown to alter the optical properties of pre-cancerous and cancerous tissue. Light based detection systems identify these "optical signatures" created during tissue transformation to provide a real-time assessment of tissue structure and metabolism. One of such optical technologies is called "Autofluorescence Spectroscopy "which is an emerging most potential and dynamic tool in the early diagnosis of oral cancer.[3]

History

The phenomena of fluorescence dates back to 1845 when Sir John Frederick William Herschel observed celestial blue colour fluorescence from a solution of quinine sulphate (in tartaric acid) in sunlight which was later published in Philosophical Translation of the Royal Society of London. Later in 1852, G.G Stokes coined the word fluorescence from fluor-spar and was the first person to propose the use of fluorescence as an analytical tool in a lecture in 1864 "On the application of the optical properties to detection and discrimination of organic substances". R. Meyer in 1897 proposed the name "fluorophores" to describe endogenous compounds or specific functional groups responsible for fluorescence.[4]

Gregorio Weber was elected to the National Academy Of Sciences in 1975 for his significant contributions to the fields of fluorescence spectroscopy.

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Principle

Autofluorescence Spectroscopy utilize spectroscopic principles - When light interacts with biological tissue, there is excitation and re-emission of light of varying colors, which can be detected by sensitive spectrometers. [5]As light illuminates the targeted tissue, biomolecules, termed fluorophores, absorb the energy in the illuminating light and respond by emitting fluorescent light of lower energy (and longer wavelength).
Known fluorophores include

Components of the connective matrix

Metabolic coenzymes (NADH, FAD, FMN)

Aromatic amino acids

Byproducts of the heme biosynthetic pathway and lipopigments As compared to normal tissues, diseased tissues contain intrinsic fluorophore of different morphohistologic characteristics. Each group of fluorophores will respond to specific excitation wavelengths, and in turn, emit a different range of wavelengths resulting in a spectral pattern By analyzing these spectra, the physical and chemical properties of a tissue can be evaluated and it can be used to compare the autofluorescence spectra of diseased and normal tissues. [6]

General Requirements

The system consist of a light source usually in the near UV to visible wavelength range that excites the tissue through a hand held fibre optic probe attached to Fluoromax-2 spectrofluorometer. The excitation light was provided by a monochromator with 150-W ozone free Xenon lamp.Specific wavelengths required for the study were obtained by the computer guided programme. [7]The excitation light was guided to the desired site by one arm of Y-type quartz fibre bundle of fibre optic probe .The resulting emission fluorescence was collected by another arm of the fibre bundle and sent to the photomultiplier detector. The signal is then amplified and displayed in the computer monitor.

Excitation-Emission Spectra

Thoroughly disinfected fibre optic probe was held over the desired mucosal site for recording the excitation and emission spectra. Ultraviolet light at 280 and 330 nm wavelengths were used for recording the excitation spectra. Ultraviolet light at 340 and 390 nm wavelengths were used for recording the emission spectra. The resulting emission and excitation spectra were recorded from 350 to 600 nm in 1 nm increments. [8]

At a wavelength of 630 nm, endogenous autofluorescence has been noticed in tumor cells. This fluorescence is in association with porphyrins. The emission band at 400-405 nm is mainly due to the presence of collagen when excited at a wavelength of range 325-360 nm, while emission at 440-460 nm can be attributed to the presence of nicotinamide adenine dinucleotide (NADH) at an excitation wavelength of 290 nm. Also, 400-405 nm excitation is for porphyrin with emission at 630-690 nm and emission at 350 nm is for tryptophan with an excitation wavelength at 280 nm. [9]

Classification of Different Lesion Types

For 410 nm excitation, the average ratio of the fluorescence intensity at the peak intensity centered around 490 nm of contralateral normal mucosa to that of malignant lesions was 6, while for benign lesions this ratio was less than 2. Also, they noticed a porphyrin-like peak at 640 nm under excitation with 410 nm for the neoplastic lesions, while this peak did not occur in healthy mucosa or benign lesions. [10]Using these two characteristics in a scatter plot and drawing a straight line between neoplastic lesions on the one hand and benign and healthy mucosa on the other, they achieved fairly good separation.

Advantages

1. Simple to use and non-invasive.
2. No consumable reagents needed
3. Provides real time results.
4. Can be performed by a wide range of operators after short training (Moderately technique sensitive).
5. Limited operator variability.
6. High sensitivity for any oral mucosal disorder.
7. Not time consuming.
8. Utilize spectroscopic principles to capture fluorescence emission spectra from a larger tissue sample area than is possible with point spectroscopy.
9. Provides two dimensional information allowing for the detection of lesion specific features such as homogeneities.

CONCLUSION

Early detection of oral premalignant and malignant lesions is the most effective step of improving the survival rate of oral cancer patients. The main purpose of autofluorescence spectroscopy is to highlight oral lesions and to assist the physicians to locate better the surgical margins. Autofluorescence techniques can only act as an adjuvant and cannot be used as a confirmatory test in the diagnosis of oral cancer. As the technology and techniques evolve, these modalities progressively reduce the need for conventional biopsy techniques, can define the surgical margins, and may emerge as a powerful chair side diagnostic tool for oral cancer.

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