Bio-Optical Imaging: An Advanced Cancer Detection Modality
Pramod John, Jayasree VM

Abstract
Oral cancer is one of the most common malignancies that affect human beings across the world, mainly because of the widespread consumption of tobacco and its products. Tobacco and its products have been implicated as predisposing factors in the etiology of oral cancer. The commonest type of oral cancer is oral squamous cell carcinoma. Early detection is very important in the management of oral cancer. However, when the lesion is detected in the oral cavity, it is very much advanced for effective management with minimum morbidity and mortality. It is clearly known that cancer and cancer treatment are always associated with considerable morbidity and mortality, especially because of the late detection. In order to improve the clinical outcome, early detection is very important. Though the oral cavity is easily accessible for direct visual examination, medical practitioners mostly neglect this area in the general examination of the patient. The general dental practitioners also do not give much importance to the oral mucosal changes while carrying out dental treatment. One of the emerging technologies in the early detection of oral cancer is the use of non-invasive in vivo tissue imaging that capture the molecular changes at high-resolution to improve the detection capability of early stage oral cancer. Various newer cancer detection methods currently available are the use of optical imaging such as autofluorescence, fluorescence diagnosis, laser confocal endomicroscopy, surface enhanced Raman spectroscopy, optical coherence tomography and confocal reflectance microscopy in the early oral cancer. Fluorescence diagnosis is emerging as a promising method in the differentiation of cancerous lesions from benign lesions, thus helping in the determination of resolution for the surgical resection of affected area of malignancy very accurately. Laser confocal endomicroscopy offers in vivo cellular imaging of tissue structures from surface to subsurface layers and can be used as a minimally invasive biopsy technique for the early detection of the lesion. Based on the pattern of the spectrum obtained from the saliva, the detection of the lesion is possible in surface enhanced Raman spectroscopy. Optical coherence tomography can be used in the imaging of histopathological features of oral lesions. The above mentioned techniques can help in defining the surgical margin and in the assessment of successful management of the lesion. The review process was carried out by literature search using appropriate key words for articles published between 1995 1nd 2015. Out of 4,46,000 articles obtained, 1480 articles were selected. In the final review process, 179 articles were selected. After subjecting the articles to systematic review, finally 19 articles were selected. The study designs in the articles were interventional experimental studies.

Keywords: Confocal Endomicroscopy; Confocal Reflectance Microscopy; Fluorescence; Malignancy; Oral Cancer; Optical Imaging; Optical Coherence Tomography; Raman Spectroscopy.

Introduction
The global incidence of oral cancer is increasing at an alarming proportion, mainly because of the widely prevalent use of tobacco and tobacco products. Approximately about 300,000 new cases are reported every year globally and the number of deaths due to oral cancer is about 127,654. The mortality rate due to oral cancer is about 50%. Oral cancer is the sixth most common cancer affecting human beings. Though the oral cavity is easily accessible for visual examination, the lesion is very much advanced when detected. Early detection of oral cancer and instituting appropriate treatment are the effective cancer management measures to prevent the morbidity and mortality. Lack of expertise in differentiating benign and malignant lesions is also a significant reason in the failure of detection of oral cancer, especially by the general dental practitioners and the medical practitioners. Clinical examination, toluidine blue vital staining and biopsy techniques are the main investigative methods in the detection of oral squamous cell carcinoma (OSCC). Since diagnosis with biopsy procedures takes time, it is appropriate to opt for other non-invasive diagnostic modalities.
Oral carcinogenesis is a multi-step process, during which various structural, molecular, biochemical and biological changes occur in the epithelium and stroma that alter the biological and optical properties of dysplastic and malignant tissue. These changes can be detected at an early stage itself by using optical principles such as endogenous autofluorescence and exogenous fluorescence. Autofluorescence makes use of the intrinsic fluorescing ability of biomolecules such as nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD) in epithelial layers and elastin and collagen in the stroma following excitation using ultraviolet (UV) rays or visible light of appropriate wavelength. Qualitative and quantitative changes due to oral carcinogenesis in the native fluorophores can be detected by capturing its emission signal. Other than the fluorophores, fluorescence signals can also be obtained by adding external fluorophores such as 5-aminolevulinic acid (5-ALA), hypericin etc. Optical imaging can be achieved using molecular imaging probes such as gold nanoparticles, iron oxide nano rings and other biomarker targeted conjugates. Apart from that, endoscopic optical imaging methods like laser confocal endomicroscopy can also be used and optical coherence tomography (OCT) can be used to obtain in vivo high resolution imaging of oral epithelial tissues. As the cancer cells possess certain biomarkers, they can be detected by using surface enhanced Raman spectroscopic (SERS) imaging that analyzes particular scattering signal from specific antibody conjugated gold nanoparticles labeled with highly SERS efficient reporter tags. Confocal reflectance microscopy (CRM) is a non-invasive technique for obtaining visible image of the tissue.

Methods of Literature Search
A systematic review of literature was performed to find out the published and online articles available on bio-optical imaging in a time frame between 1995 and 2015. The key words used in the search process were oral cancer, potentially malignant lesions, bio-optical imaging, fluorescence studies, Raman spectroscopy, autofluorescence and non-invasive biopsy procedures. In the initial search process 4,46,000 articles were obtained and after performing systematic review, 1480 articles were selected. Out of 1480 articles, 179 articles were chosen for the review process. Based on the strict quality evaluation protocols, 19 key articles were selected for the final review. The citation indices searched were Web of Science, Embase, PubMed and Google search. The titles and abstracts of the published article on bio-optical imaging were checked rigorously to make sure that the data sources are clearly identified. The study design in the selected articles was interventional and experimental studies.

Records identified through database searching (4,46,000 results)
↓
Total unique peer reviewed articles eligible for screening that were published in English language, using an adult population in a trial or empirical study (1480 results)
↓
Number of studies examined for inclusion criteria (179 results)
↓
Studies examined for inclusion criteria 19 results

Molecular Imaging Probes
Molecular imaging can image and quantify molecular alterations of malignant cells even at an early stage. Thus, it can help in the detection and staging of the tumor as well as monitoring of the therapeutic efficacy or response of the tumor cells to treatment. This is achieved by using a labeled probe for detecting, a ligand having high affinity and specificity to the target and a method to amplify the signal from the label and a high resolution imaging system to detect the label. Due to the optimum physical and chemical properties, gold nanoparticles are used in cellular imaging. Antibody conjugated gold nanoparticles can target and illuminate cancer cells under reflectance-based optical imaging system. This is achieved because of the optical contrast between the normal and malignant cells. Gold nanoparticles can also be used as multifunctional SERS nanosensors to probe cellular chemistry at subendosomal resolution. Out of various nanostructures, gold nanoshells are very effective contrast agents for optical imaging.

Molecular imaging methods also help in evaluating angiogenic responses and therapeutic angiogenesis. Vascular endothelial growth factor (VEGF) signaling pathway is very important in the development of the normal vasculature and
many pathological conditions. This is the main target for many emerging anti-angiogenic therapies. Many of the aggressive malignancies express VEGF and the measurement of its levels can help in cancer therapy.

Auto fluorescence

As already stated, when the oral tissue is illuminated with UV light, fluorophores absorb some of the portions of the photons and become excited and start emitting lower energy photons that can be detected as fluorescence of the mucosal surface. Presence of malignancy can alter the concentration and fluorescence emitting properties of these fluorophores. Detection of this change can help in the detection of the severity of the lesion, thus helping in the clinical assessment of the lesion without resorting to any invasive procedure. This analysis can be done either by obtaining the autofluorescence analysis or autofluorescence imaging. The spectral analysis or autofluorescence spectroscopy can also help in the classification of the lesion. The fluorescence emitted can also be captured using a charge-coupled device (CCD) camera (autofluorescence imaging).

Onizawa et al., and Dinish et al., in their study, found fluorescence of malignant lesions between orange and red band of the spectrum. This proved that the blood components with protoporphyrin are the causes for autofluorescence. The intensity of fluorescence increases in accordance with the tumor progression. It has also been found that the subepithelial stromal collagen fibers are the origin or the source for autofluorescence. Studies have shown that in the oral mucosa, green fluorescence is due to collagen degradation and red fluorescence is due to porphyrin. Poh et al. illuminated oral tissues using a device called VELSscope and demonstrated autofluorescence. In this study, loss of green fluorescence was attributable to the malignant change taking place at the cellular level.

Fluorescence Diagnosis

Fluorescence diagnosis (FD) using fluorescence endoscopy system is used to visualize the malignant lesion in an organ system after applying a tumor-selective photosensitizer either topically or systemically. Though many photosensitizers have been evaluated, 5-aminolevulinic acid (5-ALA) has been found to be very effective in the diagnosis of oral cancer. It is a precursor in the heme biosynthetic pathway of nucleated cells. It is metabolized by endogenous enzymes to produce protoporphyrin IX (PPIX). It is an endogenous photosensitizer.

Laser Confocal Endomicroscopy

Laser confocal endomicroscopy is an optical technique that helps in the in vivo imaging of tissue structures from surface to subsurface layers down to a few hundred micrometers. For the detection of oral malignant lesions, fluorophores such as fluorescein and hypericin and 5-ALA (which is a fluorescent precursor) could be used. Laser confocal endomicroscopy system makes use of a low-powered laser (usually an argon-ion laser generating an excitation wavelength of 488 nm). It is focused to a single point in a defined microscopic field of view and the same lens is used as condenser and objective folding path. The point of illumination is same as that of the point of detection within the specimen. The illumination and detection systems are in the same focal plane and are termed “confocal”. As fluorescence has to be produced, a fluorescent dye is used as a contrast agent to make the objects visible. The detected signals from the illuminated spot are captured and measured. The image of the scanned region is constructed and digitized. This helps in detecting fine cellular and subcellular structures.
image viable tissue with high resolution and contrast without having to perform an invasive biopsy procedure. Using the device different areas can be scanned. The image thus obtained helps in the identification of malignant cells. Thus, CRM is a non-invasive biopsy technique without the need of any surgical procedure, tissue sectioning and microscopic evaluation\textsuperscript{8,19}.

Conclusion

Early detection of cancer is very important in the overall management of potentially malignant lesions as well as oral cancer. Instituting early detection helps in the possible complete cure of the lesion. As several newer, sensitive and non-invasive techniques are available, the early cancer detection must be made more effective involving a larger number of populations who are especially at a greater risk of developing cancer.

Author Affiliations

1. Dr. Pramod John, Professor and Head, Department of Oral Medicine and Radiology, Kannur Dental College and Hospital, Anjarakandy, 2. Dr. Jayasree VM, Senior Lecturer, PSM Dental College, Thrissur, Kerala State, India.

References


Corresponding Author
Dr. Pramod John,
Professor and Head,
Department of Oral Medicine and Radiology,
Kannur Dental College and Hospital,
Anjarakandy, Kerala State, India.
Ph: +91 9447140774
Email: pramodjohn@hotmail.com

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