Review Article

JRAD Journal of Research and Advancement in Dentistry

J Res Adv Dent 2016;5:1:350-356.

Bio-Optics: A Boon in Early Detection of Oral Cancer

Raghav Kumar[1] Smriti Singh[2*] Trisha Rastogi[3] Sugandha Sharma[4]

[1]Professor and Head, Department of Oral Medicine and Radiology, D.J College of Dental Sciences and Research, Modinagar, India.
[2]PG Student, Department of Oral Medicine and Radiology, D.J College of Dental Sciences and Research, Modinagar, India.
[3]Senior Lecturer, Department of Oral Medicine and Radiology, D.J College of Dental Sciences and Research, Modinagar, India.
[4]Senior Lecturer, Department of Oral Medicine and Radiology, D.J College of Dental Sciences and Research, Modinagar, India.

ABSTRACT

Background: Oral cancer is a significant and a major health concern in developing countries and worldwide. Early detection and treatment of the cancer should be necessary for good prognosis and to increase survival rates. A number of newer advancements were emerged to boost the conventional oral examination procedure for early detection and intervention of oral cancer. The most recent ones are bio-optics.

Keywords: Bio-optics, Oral cancer, Diagnosis, imaging.

INTRODUCTION

Oral squamous cell carcinoma or Oral cancer is any malignancy tissue growth present in the oral cavity. It is the 8th most cancer worldwide with 274,000 new cases reported yearly. The majority individuals are from the developing countries. In India oral cancer ranks first position in males and third among females. Recent data suggests that in south central Asia oral cancer ranks in third position[1]. The most common risk factors are usage of tobacco and alcohol. Recent epidemiological data shows that prevalence tongue cancer increases among young adults in India and worldwide. Etiology may include use of smokeless tobacco, drug abuse, geographic conditions and HPV[2].

Despite of advancements in the treatment of oral cancer the survival rate and prognosis of the individuals was poor. Early detection or diagnosis is strongly required to increase the survival rate and to decrease the treatment associated morbidity. In Developed countries like USA, there is only marginal improvement in the 5 year survival rate for oral cancer since 1975. This improvement is due to earlier diagnosis and advancements in treatment. In developing countries diagnosis of oral cancer is found to be done at later stages when compared to developed countries. Thus an early detection and diagnosis of oral cancer is necessary[3].

The present review is aimed to give a brief outline about the traditional diagnostic methods and emphasizing on the recent advancements mainly non invasive methods occurred in the early detection of oral cancer.

TRADITIONAL METHODS IN DIAGNOSIS OF ORAL CANCER

The standard method for screening is oral examination and palpation performed by general dentists or general physicians.

Visual inspection is done by white light illumination followed by palpation of lesion at suspected site. The adjuncts used are toluidine blue, ViziLite plus, a disposable chemiluminescent light source emitting blue light. Studies (Farah and Mc Cullough[4], Ram and Siar[5]) showed that vizilite did not differentiate between inflammatory or benign and premalignant or malignant oral lesions. So it is necessary to develop alternate diagnostic modalities which can

Received: May. 18, 2015: **Accepted:** Aug. 23, 2015
*Correspondence Dr. Smriti Singh.
Department of Oral Medicine and Radiology, D.J College of Dental Sciences and Research, Modinagar, India.
Email: ssdoctor2010@gmail.com

pISSN 2278-0076
eISSN 2321-9270

enhance the examination of oral lesions and helps in discriminating benign and premalignant lesions.

EMERGING TECHNOLOGIES IN DIAGNOSING ORAL LESIONS

Non invasive in vivo tissue imaging is using to see the changes in molecular level with high resolution to improve the detection capability of the lesion at an early stage. The following are some of the emerging technologies of which the following review is going to discuss. The following are[6]:

1) Laser confocal endomicroscopy (LCE)
2) Surface enhanced Raman spectroscopy (SERS)
3) Elastic scattering spectroscopy
4) Nuclear magnetic resonance spectroscopy
5) Confocal reflectance microscopy (CRM)
6) Optical coherence tomography
7) Angle-resolved low coherence interferometry

LASER CONFOCAL ENDOMICROSCOPY (LCE)

Principle: Confocal microscopy is an adaptation of conventional optical microscopy, in which the illumination from a laser source is associated with a pinhole in such a way as to geometrically eliminate information lying outside the focal plane. The resulting image is an optical section taken at a specific depth from the surface, the width of which depends on the confocal technology[7]. In vivo confocal fluorescence endomicroscopy permits the characterization of microstructures and cellular details. It is based either on auto-fluorescence from the tissue, or on the administration of exogenous dyes[8]. Five major fluorescent dyes are used for clinical head and neck imaging. Dyes like fluorescein, acriflavine, proflavine, hypericin and 5-aminolevliniv (5-ALA) acid.

Recently, Haxel et al[9]. extended the use of confocal endomicroscopic imaging to five regions of the human oropharynx, namely the buccal area, the tongue, the base of the tongue, the tonsils, and the floor of the mouth. In vivo imaging was carried out using intravenously injected fluorescein while the dye acriflavine was used for ex vivo imaging of SCC tissue. Their results showed that confocal endomicroscopy is suitable for epithelial evaluation of the anterior parts of the oral cavity, including the buccal area, tongue and floor of the mouth. The technique however was not suitable for application on the tonsils or the base of the tongue as the procedure triggered pharyngeal reflexes in the subject's examined[9].

The disadvantages of confocal endomicroscopy include the fact that it is an examiner-dependent technology and thus hands-on training is of crucial importance before reliable histological diagnosis can be made[6].

SURFACE ENHANCED RAMAN SPECTROSCOPY (SERS)

Raman spectroscopy is being investigated as a diagnostic tool for characterizing cancer cells and early malignant changes and distinguishing these cells from normal cells. It has a distinct advantage over other optical techniques: it provides information on molecular composition and structure of living tissue[10]. Resonant response can greatly enhance the efficiency of Raman scattering, typically by up to 10^6 to 10^7 fold. Further improvement in the enhancement factor by a factor of 10^4 to 10^5 is also possible if the molecules are sandwiched in between particles within an aggregate, giving the possibility of single-molecule spectroscopy[11].

Oliveira et al[12]. demonstrated the changes in the vibrational bands of normal, dysplastic (DE) and squamous cell carcinoma (SCC) tissues that seem to arise from the compositional, conformational and structural changes of proteins. Huang et al compared SERS spectra from non-malignant to malignant cells and observed that malignant cells have different chemical composition in cell membrane than non malignant cells[6]. Guze et al[13]. reported 100% sensitivity and 77% specificity for differentiating premalignant and malignant oral lesions from normal mucosa and benign lesions in humans by Raman spectroscopy.

ELASTIC SCATTERING SPECTROSCOPY (ESS)

It uses analytical and objective statistical method by subjective interpretation of images. It provides optical geometrical information that is based on white light reflectance[14]. The ESS method senses micro-morphology changes at the level of sub-cellular architectural changes, such as nuclear grade, nuclear to cytoplasmic ratio, mitochondrial

size and density, without actually imaging the microscopic structure. Because ESS detects changes at a sub-cellular level, it supplies information that may not be provided by conventional histology. Thus, ESS provides an optical signature of a tumour that greatly depends on that tumor's morphology[15].

ESS covers 300-900 nm ranges and pulsed xenon arc lamp as light source. The system has two fibreoptic probes, one for transmitting light into the tissue and the other for collecting scattered light. The tip of the collecting probe is placed in direct contact with the lesion and a background measurement taken; the lamp is then activated. The entire measurement processing display takes less than 1 second[16].

Muller et al[17]. used three spectroscopic techniques to assess 91 tissue sites from 15 patients with varying degrees of oral malignancy (normal, dysplastic, and cancerous sites) and eight healthy volunteers. They described this method as trimodal spectroscopy and reported a sensitivity and specificity of 96% and 96%, respectively, in distinguishing cancerous/dysplastic (mild, moderate, and severe) from normal tissue. ESS was able to distinguish dysplastic from cancerous cells with a sensitivity of 64% and specificity of 90%.

A recent development is differential path-length spectroscopy which measures scattered photons in predetermined path lengths. It has fixed photon visitation depth, path length, and absolute measurement of absorbers[14].

Amelink et al[18]. used differential path-length spectroscopy to study 76 spectra (45 nondysplastic and 31 dysplastic) collected from 27 leukoplakias. Based on a combination of the three variables of blood oxygenation, vessel diameter, and blood volume fraction, nondysplastic and dysplastic leukoplakias can be discriminated with a sensitivity and specificity of 91% and 80%, respectively.

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

It uses the magnetic properties of atomic nuclei to determine physical and chemical properties. It allows 3D study of atoms in molecules. NMR has been used to identify metabolic signatures of OSCC compared with normal tissues. Clinical studies have confirmed that the choline/creatine ratio is significantly higher in OSCC than in normal tissue. Over expression of glucose transporter 1 has been reported in OSCC[19]. More research and studies should be done using this technique to determine the sensitivity and specificity for diagnosing oral lesions.

CONFOCAL REFLECTANCE MICROSCOPY (CRM)

In vivo confocal reflectance microscopy is based on the backscattered light from cellular structures, which provide the image signal. In this mode, the image contrast results from variations in the refractive index of the cellular components highlighting the cellular morphology[20]. CRM is an optical method of noninvasively imaging tissue without fixation, sectioning, and staining as in standard histopathology thus has the potential for further development and use in clinical practice for pre-surgical determination of cancer margins and intra surgical guidance on the patients in real time. This it can act as a diagnostic tool for the early detection of oral cancer and precancer[6].

OPTICAL COHERENCE TOMOGRAPHY (OCT)

It is a non invasive imaging technique of tissues that produce cross sectional images with high spatial resolution of 10 to 20 μm. OCT is based on Michelson interferometer configuration. It was introduced in 1988 for studying structures of hard and soft tissues of the oral cavity[21]. Hamster cheek pouch model was used to study the epithelial and sub-epithelial changes in oral carcinogenesis. The new advancement in OCT is Optical Doppler Tomography used in quantification of velocity of blood flow in detecting vascular changes in carcinogenesis[22].

In vivo 3-D OCT was available to visualize the detailed histological features of the oral lesions with an imaging depth down to 2–3 mm. The comprehensive diagnostic images can be used to define surgical margin and to provide a direct assessment of the therapeutic effectiveness. Furthermore, OCT system can be readily combined with nonlinear optical modalities, such as two-photon excited fluorescence (TPF) and second-harmonic generation (SHG). The combined techniques were found to yield increased sensitivity and specificity in the diagnosis of oral dysplasia and

malignancy[23]. However, there are several issues related to the use of OCT for clinical studies. These include the fact that[24]:

(i) Histopathologist is required to interpret and assess the live image. interpretation and assessment of the acquired live histology images;

(ii) As the images do not provide quantitative information, visual assessment would be needed, which is subjective;

(iii) Due to the small size of the OCT probe, only a very small area can be examined at a time. Therefore, the detection of functional and structural changes at a precancerous stage still remains a challenge for OCT.

ANGLE-RESOLVED LOW COHERENCE INTERFEROMETRY

An emerging biomedical imaging technique uses the properties of scattered light to measure average size of different cell structures, cell nuclei, and sub-cellular features of epithelial tissues up to 500 μm below the surface. It performs an objective analysis of tissue and delivers direct confirmation of precancerous disease to the physician[25]. Overall, the combined studies showed 91% sensitivity and 97% specificity for detecting dysplasia, using histopathology as the standard[26].

The technology shows promise as a clinical tool for in situ detection of dysplastic or precancerous tissue. Wax et al. and Terry et al. reported sensitivity of this technology in diagnosis of esophageal lesions of 100% and specificity 85%[27]. Above mentioned studies are taken in tissues other than oral cavity, in our literature search we did not find any studies in oral cavity.

Table 1: Some of the important points and techniques regarding optical biopsy are summarized.

Optical Biopsy	Technology	Light source	Information provided	Sensitivity %	Specificity %
Auto-fluorescence Spectroscopy	Fluorochromes fluorescence (NAD, FADH)	ultraviolet and visible spectrum light	Distinguish malignant tissue by concentration of (NAD, FADH), re-emit green light	81	100
Enhanced dye fluorescence	Fluorochromes fluorescence (protoporphyrin IX)	ultraviolet and visible spectrum light	Distinguish malignant tissue by high concentration of (protoporphyrin IX), re-emit red light	100	100
Ratio imaging	fluorescence (protoporphyrin IX, NAD, FADH)	ultraviolet and visible blue light	Compare a ratio of red emission of (protoporphyrin IX) from malignant cells with the green emission from normal	From 60 to 97	From 75 to 99
Raman spectroscopy	Raman vibrational spectroscopy	laser-based spectroscopic technique	enabling chemical characterization	80.5	86.2
Elastic scattering spectroscopy	Elastic scattering (white light reflectance)	pulsed xenon arc lamp	provides optical geometrical information	92	79

Differential path-length spectroscopy	Elastic scattering (white light reflectance)	tungsten-halogen lamp	cell biochemistry, intracellular morphology and micro vascular properties such as oxygen saturation and average vessel diameter	69	85
Optical coherence tomography	scattered light (Fourier domain mode lock swept source-based) OCT	laser-based	Provide high-speed three dimensional OCT pictures	Subjective image required interpretation	Subjective image required interpretation
Angle-resolved low coherence interferometry (A/LCI)	scattered light to measure the average size of different cell structures	laser-based	delivers direct confirmation of precancerous disease to the physician	100	85

CONCLUSION

In our literature search we find very few studies using different techniques of bio-optics imaging in India (Sahu et al[28], Singh et al[29], Singh et al[30], Deshmukh et al[31], Malini et al[32], Krishna et al[33]). As these techniques need a trained technician to interpret the images, technique sensitive procedures may be the reasons for not using this technology for diagnosing oral lesions although they were popular in medical sciences. As these are non invasive procedures so necessary steps should be taken to encourage the bio-optics imaging for early detection and diagnosing the oral lesions.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

REFERENCES

1) Masthan. K. M. K, Babu. A. N, Shanmugam. K. T, Jha. Abhinav. Nanotechnology- its application in oral cancer. JCDR, 2012 September (suppl), Vol-6(7): 1328-1330.

2) Messadi. D. V. Diagnostic aids for detection of oral precancerous conditions. International Journal of Oral Science (2013) 5, 59–65.

3) Shin. D, Vigneswaran. N, Gillenwater. A, Kortum. R. R. Advances in fluorescence imaging techniques to detect oral cancer and its precursors. *Future Oncol*. 2010 July; 6(7): 1143–1154.

4) Ram S, Siar CH. Chemiluminescence as a diagnostic aid in the detection of oral cancer and potentially malignant epithelial lesions. Int J Oral Maxillofac Surg 2005; 34(5):521–527.

5) Farah CS, McCullough MJ. A pilot case control study on the efficacy of acetic acid wash and

chemiluminescent illumination (ViziLite™) in the visualization of oral mucosal white lesions. Oral Oncol 2007; 43:820–824.

6) Olivio. M, Bhuvaneswari. R, Keogh. I. Advances in bio-optical imaging for the diagnosis of early oral cancer. Pharmaceutics 2011, 3, 354-378.

7) Minsky M. Memoir on inventing the confocal scanning microscope. Scanning 1988; 10(4):128–38.

8) Abbaci. M, Breuskin. I, Casiraghi. O, De Leeuw. F, Ferchiou. M, Temam. S, Laplace-Builhe. C. Confocal laser endomicroscopy for non-invasive head and neck cancer imaging: A comprehensive review. Oral Oncology 50 (2014) 711–716.

9) Haxel, B.R.; Goetz, M.; Kiesslich, R.; Gosepath, J. Confocal endomicroscopy: a novel application for imaging of oral and oropharyngeal mucosa in human. Eur. Arch. Otorhinolaryngol. 2010, 267, 443-448.

10) Witjes MJH. Raman spectroscopy in clinical diagnosis of head & neck pathology. Head & Neck Oncology. 2009; 1 Suppl 1:08.

11) Rhodus NL, Ho V, Miller CS, Myers S, Ondrey F. NF-kappa B dependent cytokine levels in saliva of patients with oral preneoplastic lesions and oral squamous cell carcinoma. Cancer Detect Prev. 2005; 29:42–5.

12) Arellano-Garcia ME, Hu S, Wang J, Henson B, Zhou H, Chia D, et al. Multiplexed immunobead-based assay for detection of oral cancer protein biomarkers in saliva. Oral Dis. 2008; 14:705–12.

13) Guze K, Pawluk HC, Short M, Zeng H, Lorch J, Norris C, et al. Pilot study: Raman spectroscopy in differentiating premalignant and malignant oral lesions from normal mucosa and benign lesions in humans. Head Neck. 2014 Feb 15. doi:10.1002/hed.23629.

14) Omar. E. Current concepts and future of non invasive procedures for diagnosing oral squamous cell carcinoma- a systematic review. Omar Head & Face Medicine (2015) 11:6 DOI 10.1186/s13005-015-0063-z.

15) Guze K, Pawluk HC, Short M, Zeng H, Lorch J, Norris C, et al. Pilot study: Raman spectroscopy in differentiating premalignant and malignant oral lesions from normal mucosa and benign lesions in humans. Head Neck. 2014 Feb 15. doi:10.1002/hed.23629.

16) Upile T, Jerjes W, Sterenborg HJ, El-Naggar AK, Sandison A, Witjes MJ, et al. Head & neck optical diagnostics: vision of the future of surgery. Head Neck Oncol. 2009; 1:25. doi: 10.1186/1758-3284-1-25.

17) Muller MG, Valdez TA, Georgakoudi I, Backman V, Fuentes C, Kabani S, et al. Spectroscopic detection and evaluation of morphologic and biochemical changes in early human oral carcinoma. Cancer. 2003; 97(7):1681–92.

18) Amelink A, Sterenborg HJ, Bard MP, Burgers SA. In vivo measurement of the local optical properties of tissue by use of differential path-length spectroscopy. Opt Lett. 2004; 29(10):1087–9.

19) Maheshwari SR, Mukherji SK, Neelon B, Schiro S, Fatterpekar GM, Stone JA, et al. The choline/creatine ratio in five benign neoplasms: comparison with squamous cell carcinoma by use of in vitro MR spectroscopy. Am J Neuroradiol. 2000; 21:1930–5.

20) Rajadhyaksha M, Grossman M, Esterowitz D, Webb RH, Anderson RR. In vivo confocal scanning laser microscopy of human skin: melanin provides strong contrast. J Invest Dermatol 1995; 104(6):946–52.

21) Colston, B.W., Jr.; Everett, M.J.; Da Silva, L.B.; Otis, L.L.; Stroeve, P.; Nathel, H. Imaging of hard- and soft-tissue structure in the oral cavity by optical coherence tomography. Appl. Opt.

1998, 37, 3582-3585.

22) Wilder-Smith, P.; Krasieva, T.; Jung, W.G.; Zhang, J.; Chen, Z.; Osann, K.; Tromberg, B. Noninvasive imaging of oral premalignancy and malignancy. J. Biomed. Opt. 2005, 10, 051601.

23) Clark, A.L.; Gillenwater, A.; Alizadeh-Naderi, R.; El-Naggar, A.K.; Richards-Kortum, R. Detection and diagnosis of oral neoplasia with an optical coherence microscope. J. Biomed. Opt.

2004, 9, 1271-1280.

24) Balas, C. Review of biomedical optical imaging—a powerful, non-invasive, non-ionizing technology for improving in vivo diagnosis. Meas. Sci. Technol. 2009, 20, 104020.

25) Mayinger B, Horner P, Jordan M, Gerlach C, Horbach T, Hohenberger W, et al. Light-induced autofluorescence spectroscopy for the endoscopic detection of esophageal cancer. Gastrointest Endosc. 2001; 54(2):195–201.

26) Wax A, Pyhtila JW, Graf RN, Nines R, Boone CW, Dasari RR, et al. Prospective grading of neoplastic change in rat esophagus epithelium using angle resolved low-coherence interferometry. J Biomed Opt. 2005; 10(5):051604.

27) Terry NG, Zhu Y, Rinehart MT, Brown WJ, Gebhart SC, Bright S, et al. Detection of dysplasia in Barrett's esophagus with in vivo depth-resolved nuclear morphology measurements. Gastroenterology. 2011; 140(1):42–50.

28) Sahu A, Sawant S, Mamgain H, Krishna CM. Raman spectroscopy of serum: an exploratory study for detection of oral cancers. Analyst. 2013; 138(14):4161–74. 29) 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; 10:105002.

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(14):4175–82.

31) 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(12):127004.

32) 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(3):179–93.

33) 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(9):1128–35.