An update on light-based technologies and fluorescent imaging in oral cancer detection

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Abstract:

Oral cancer is a potentially fatal disease that constitutes an important portion of tumors of head and neck region. More than 90% of oral cancers are oral squamous cell carcinomas (OSCC). Regardless of the fact that the oral cavity is easily accessible to the accumulation of carcinogens, most oral cancers are typically detected at an advanced stage leading to lower survival rate among subjects. Therefore, early detection of the oral cancer and its precursor lesions may be the most effective means to improve prognosis and treatment in most patients. One of the emerging technologies is the use of optical methods to capture the molecular changes at high-resolution to improve the detection capability of early stage disease. Optical diagnosis techniques when compared with traditional approaches such as vital staining, biopsy has many advantages such as objectivity, speed, and cost. Moreover, these incorporate particularly noninvasive methods of oral cancer detection. The present article highlights various optical methods and their role in oral cancer detection.

Key words:

Biopsy, fluorescence, optical, oral cancer, potentially malignant disorders

Introduction

Oral cancer or oral squamous cell carcinomas (OSCC) constitutes cancers of the lip and oral cavity. Oral cancers are regarded as the sixth most common type of cancer worldwide.[1] Oral cancer has a 5-year mortality rate of approximately 50%,[1] which has not changed significantly over the last 50 years.[2] Oral cancer is considered to account for an estimated 650,000 new cancer cases and 350,000 cancer deaths worldwide per year.[3] High risk regions for the disease are known, including South-Central Asia for cancers of the oral cavity, and South America and Western Asia for laryngeal cancers.[3]

It is well established that the major risk factors for oral cancer are tobacco and alcohol consumption constituting approximately 75% of cases. Among consumers of both products, risks of oral cancer tended to combine more in a multiplicative than additive fashion and were increased more than 35-fold among those who consumed two or more packs of cigarettes and more than four alcoholic drinks per day.[3] The high morbidity rate in OSCC can be attributed to the delay in the diagnosis of the disease.[4]

In addition to tobacco and alcohol consumption, there is increasing evidence for human papilloma virus (HPV), subtype HPV-16, having a role in the cause of oral cancer in approximately 20% of oral cancers and 60-80% of Oro-pharyngeal carcinomas were considered to be attributable to HPV infection.[1]

The diagnosis of oral cancer and/or the malignant potential of an oral lesion is based on various aspects such as (a) etiology-associated with the use of tobacco, (b) presence of factors such as detection of HPV clinical appearance of the lesion (leukoplakic, erythroplakic, nodular, ulcerative, verrucous), (c) location of the lesion—the high risk sites being floor of the mouth, ventrolateral aspect of the tongue, and so on, (d) histopathological aspects—presence of epithelial dysplasia, and (e) molecular biological aspects of the lesion.[5]

At present, the diagnosis of OSCC is through comprehensive clinical examination and histological analysis of suspicious areas, but it may remain undetected in hidden sites.[6]

The conventional method for oral cancer screening includes staining procedures and palpation typically performed by general dentists or physicians; whereas, visual inspection of the oral cavity is performed under normal white light illumination of both normal tissue and suspicious lesion. Downer *et al*. has systematically reviewed the performance of visual examination for oral cancer detection and found the sensitivity values (**Sensitivity** is the ability of a test to correctly identify those with the disease) that ranged from 60 to 97% and

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the specificity values (**Specificity** is the ability of the test to correctly identify those without the disease) that ranged from 75 to 99%.[7] As general dentists lack the experience to identify early lesion development in oral cancer, any suspicious lesion may be referred to oral cancer specialists, who can frequently recognize subtle visual changes associated with early lesions.

Recently, various advancements in oral cancer research have led to the development of potentially useful diagnostic tools at the clinical and molecular level for the early detection of oral cancer. However, the gold standard for the diagnosis of oral cancer still remains tissue biopsy with histological assessment, but this technique needs trained healthcare professionals. Moreover, the technique is considered to be invasive, painful, expensive, and time consuming. Therefore, alternative noninvasive light-based screening methods have been developed for the early detection of premalignant lesions or malignancy.

Optical properties of normal oral tissue and neoplastic lesion
Recent studies have characterized the biochemical and microanatomic origins of tissue that differentiates the fluorescent properties of normal oral tissue and neoplastic lesions. The autofluorescence properties of oral tissues vary based on anatomic site and pathologic diagnosis. In normal squamous oral mucosa, autofluorescence in the UV and visible region of the spectrum is predominantly associated with collagen in the stroma. Therefore, the epithelium shows weak autofluorescence, primarily associated with mitochondrial nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD) in basal epithelial cells based on studies with MitoTracker® (Molecular Probes, Inc., OR, USA). Neoplasia is associated with a strong loss of stromal autofluorescence, which is likely to be responsible for the loss of autofluorescence observed in wide-field images. In addition, epithelial dysplasia is associated with increased mitochondrial fluorescence throughout the epithelium. Inflammatory lesions are associated with a loss of both epithelial and stromal autofluorescence.

Objectives of early detection of potentially malignant disorders (PMD) and oral cancer
The objective of early detection in oral cancer is to recognize, not only oral cancer but PMD at the earliest possible stage. Referral of these lesions to a specialist will result in an early definitive diagnosis and treatment if indicated. Even though accurately predicting malignant transformation for PMDs displaying dysplasia is not currently possible, these lesions require special attention and management strategies depending on the site, grade of dysplasia, and patient risk.[8]

The value of screening programs may not be solely limited to the detection of oral cancer. Screening opportunities should also be utilized to improve patient awareness about the relationship between risk factors such as alcohol and tobacco and oral cancer, which may play a role in prevention.[9]

Light-Based Screening Device

ViziLite
The ViziLite (Zila Pharmaceuticals, Inc., AZ, USA) system offers an alternative to white light illumination for visual examination that utilizes a disposable chemiluminescent light source that illuminates oral tissue with blue light. It involves the use of a handheld, single-use, disposable chemiluminescent light stick that emits light at 430, 540, and 580 nm wavelengths. ViziLite Plus with T Blue system requires a 30 s acetic acid pre-rinse that dehydrates the tissue.[10] The use of this light stick is intended to improve the visual distinction between normal mucosa and oral white lesions. Normal epithelium will absorb light and appear dark; whereas, hyperkeratinized or dysplastic lesions appear white. The difference in color could be related to altered epithelial thickness or to the higher density of nuclear content and mitochondrial matrix that preferentially reflects light in the pathological tissues.[11]

Studies conducted by Epstein *et al*.[12] and Kerr *et al*.[13] indicated that the ViziLite could potentially aid in the detection of oral premalignant lesions by improving brightness and sharpness. Epstein *et al*. examined 134 patients who had identified oral lesions using conventional white light and ViziLite illumination. The study showed that two lesions became clinically visible only after ViziLite examination.

The reported sensitivity of ViziLite ranged from 0[14] to 84%,[15,16] and the specificity ranged from 15[16] to 91%.[15] When ViziLite was compared with Toluidine Blue, it showed better diagnostic values; however, it did not provide additional diagnostic value compared to the conventional clinical examination.

Limitations
The principle utilized in ViziLite has limitations such as (a) low specificity, (b) not distinguishing inflammatory/benign/malignant lesions, and (c) questionable diagnostic value as there is a lack of histopathological correlation.[10]

Wide-field fluorescence imaging
It is known that clinicians illuminate tissue with white or blue light and observe light that is reflected from the mucosal surface. However, there are a range of light–tissue interactions that can be exploited to improve the visualization of neoplastic lesions. In particular, tissue autofluorescence has recently shown promise as an adjunctive diagnostic tool. Fluorophores within the oral epithelium and stroma absorb UV and visible light and can re-emit some of this light at longer wavelengths in the form of fluorescence. When the reflected illumination light is blocked with an absorbing filter, it is possible to visualize the longer wavelength fluorescence even with the naked eye. Autofluorescence originates from a variety of fluorophores in the oral cavity, and is sensitive to alterations in both tissue morphology and biochemistry associated with neoplasia.[17,18] Oral cancer and precancer display a loss of autofluorescence across a broad range of UV and visible excitation wavelengths.

Fluorescence-Based Techniques

VELscope (visually enhanced lesions scope)
The VELscope® (LED Dental, Inc., White Rock, BC, Canada) is a commercially available device to visualize tissue autofluorescence in the oral cavity. The VELscope has been

approved by the US FDA as an adjunct to traditional oral examination to enhance the visualization of oral mucosal abnormalities. The VELscope is based on the principle that abnormal tissue appears dark brown to black owing to decreased levels of autofluorescence, while normal healthy tissue emits pale green autofluorescence. Based on different studies, it is found that VELscope has a sensitivity of 98% and specificity of 100% using biopsy as "gold standard" when discriminating normal mucosa from severe dysplasia/carcinoma in situ or invasive carcinoma.[19,20]

Mehrotra et al. examined the diagnostic accuracy of the ViziLite Plus and VELscope in detecting oral dysplasia and carcinomas in 256 oral mucosal lesions deemed to be clinically innocuous based on conventional white light oral examination by an expert clinician. The authors determined the specificity and sensitivity of the ViziLite Plus and VELscope in detecting oral dysplasia and carcinomas by comparing their respective finding with the gold standard scalpel biopsy results. They reported a disappointing 0% sensitivity and 75.5% specificity for the ViziLite Plus and 50% sensitivity and 38.9% specificity for the VELscope.[14]

Advantages
The device has many advantages such that it is quick, easy to use, easy to carry, allows for broader intramural imaging, and cordless (utilizing a 12 h battery).[11] It does not require a dimmed light and can be used under incandescent light.[21]

Limitation
The major limitation of this device is its inability in discriminating high risk and low risk lesions.

Identafi 3000
The Identafi Oral Cancer Screening System uses Multi-Spectral Fluorescence and Reflectance technology to enhance visualization of mucosal abnormalities such as oral cancer or premalignant dysplasia that may not be apparent to the naked eye. The device uses white, violet, and green-amber wavelengths of light to excite oral tissue in distinct and unique ways that provides the clinician with more visual information resulting in increased confidence for recommending follow-up care.

Besides the detection of autofluorescence similar to the VELscope system, this device also examines tissue reflectance that is based on the premise of detecting changes in angiogenesis with green-amber light (540-575 nm wavelengths) illumination. The amber light is considered to enhance the reflective properties of the oral mucosa, allowing a distinction between normal and abnormal tissue vasculature. Increased angiogenesis is a known process during oral carcinogenesis and oral cancer progression. It is important to develop imaging technology for evaluating the status of tumor angiogenesis. Identafi fluorescent light makes the abnormal lesion to appear dark brown or black, and the healthy tissues reflect as blue fluorescence areas.[11]

Ayoub et al. showed sensitivity and negative predictive values equivalent to the conventional clinical study, that is, 50

and 98%, respectively. However, the specificity and positive predictive values were lower than that was reported for the conventional clinical examination, 81 versus 98% and 11 versus 50% for the specificity and positive protective value of Identafi and the conventional test, respectively.[21]

Advantages
Small, compact size coupled with an angled examination mirror allows users to easily inspect hard-to-reach areas, such as under the tongue, hard and soft palate, and back of pharynx.

Limitations
The field of illumination of the Identafi 3000 is considerably smaller than the VELscope; thus, the time required to visualize the entire oral cavity is longer. Another limitation is that the batteries will eventually need to be replaced with a freshly recharged set or a set of non-rechargeable AA batteries, if used for a long period of time, such as in a community oral cancer-screening program.

Dual-mode reflectance and fluorescence confocal microscope
Carlson et al.[22] develop this device to image molecular properties of tissue as well as tissue architecture and cellular morphology using reflective and fluorescent molecular-specific contrast agents.

The portable system uses light-emitting diode illumination to excite and collect fluorescence through a fiber bundle with no requirement for complex scanning mechanisms. Following topical application of proflavine, a surgically resected tissue specimen from the human oral cavity was imaged across the clinical margin, demonstrating qualitative and quantitative differences between normal and cancerous tissue based on subcellular image features such as nuclear-to-cytoplasmic (N/C) ratio.[22] Images obtained with the combination of reflectance and fluorescence provide information about both the morphologic and molecular changes associated with cancer progression.

Alternatively, fluorescent dyes can be applied to enhance image contrast and imaged using confocal fluorescence microscopy. Thong et al.[23] investigated the capability of a confocal fluorescence microscope system to identify morphologic details in living tongue tissue. Morphologic differences between normal and neoplastic lesions in tongue were distinguished using fluorescein and 5-aminolevulinic acid-induced protoporphyrin IX fluorescence. Confocal images obtained with exogenous contrast agents (topical acriflavine and intravenous fluorescein) showed architectural details of tissues, such as changes in nuclear size and spacing, and changes in capillary networks, providing the potential to distinguish carcinoma from normal mucosa.

Optical coherence tomography
Optical coherence tomography (OCT) is an evolving optical technology that produces cross-sectional images of tissue with a high spatial resolution of 10-20 μm.[24]

This technique is capable of imaging tissue depths of up to 1-2 mm and is thus considered suitable for imaging oral mucosal lesions. OCT employ back-scattered signals reflected from different layers within the tissue to reconstruct structural images measuring sound rather than light as in case of ultrasonic imaging. The high spatial resolution of OCT enables "optical biopsy" and provides immediate and localized diagnostic information to the clinician.[25]

In a study of 50 patients with suspicious lesions, including oral leukoplakia and erythroplakia, the sensitivity and specificity of OCT were 93-97%, showing the excellent capability of in vivo OCT for the detection and diagnosis of PMDs and oral cancer.[26]

Advances in OCT imaging

Several types of particulate contrast agent, such as airfilled microbubbles, engineered microspheres, and gold nanostructures, have been developed to improve the OCT image by enhancing the intensity of backscattered light from the tissue.[27] In addition, an OCT system can be readily combined with nonlinear optical modalities, such as two-photon excited fluorescence and second-harmonic generation. These combined techniques have been found to yield increased sensitivity and specificity in the diagnosis of oral dysplasia and cancer.[28]

Limitations

There are still limitations regarding the application of OCT: (1) a histopathologist is essential to interpret the result, which is subjective, as OCT does not provide quantitative information; (2) only a small area can be examined at a time, because of the small size of the OCT probe.[29]

Contact endoscopy

Contact endoscopy (CE) is another novel noninvasive optical diagnostic imaging method that allows in vivo and in situ examination of the cellular architecture of the superficial layers of the mucosal epithelium. Magnified images are obtained using Hopkins' rod-lens endoscope placed on the surface of the dye stained mucosal tissue. This technique allows assessment of precancerous and cancerous lesions in vivo and has significant potential in the histopathologic diagnosis of many suspicious head and neck mucosal lesions without tissue biopsy. Contact endoscopy is simply a magnifying endoscope that, when placed in direct contact with the mucosal surface, delivers images at 60 or 150 magnification. To provide contrast, methylene blue (MB) is applied topically to stain nucleic acids. Cell nuclei stained dark blue are visible against the lightly stained cytoplasm. Neoplastic cells are strongly stained by MB because of their high mitotic rate.

The first reported use of Contact endoscopy in otolaryngology head and neck surgery was by Andrea *et al*. as a diagnostic tool in the evaluation of various pathologies in the larynx in the 1990s.[30,31] They were able to visualize and diagnose laryngeal mucosal pathology from the magnification of vocal fold epithelium and microvasculature during microlaryngoscopy after staining the vocal cords with methylene blue dye.

Limitations

Some limitations of CE include the following: at high magnification, the image resolution obtained by contact endoscopy is significantly affected by glare from light reflected by cells not in focus. As a result, CE cannot provide clear images of cells beyond the most superficial layers of the epithelium, because tumor margins exist in three dimensions, which prevent the accurate distinction between carcinoma in situ and invasive carcinoma.[32]

Conclusion

Oral cancer occurs typically in males from a low socioeconomic background who smoke and consume alcohol. Patients unfortunately continue to present with late stage disease. Diagnosis of oral cancer at an early stage or at the preneoplastic level is critical to improve survival in oral squamous cell carcinoma patients. An oral screening examination is a simple noninvasive test to apply in general population. However, screening via clinical examination alone by general dentists during the patients' routine dental examination has resulted in poor detection rates. Early detection and diagnosis of oral neoplastic changes is the best way to improve patient outcomes. Conventional oral examination is based on visual inspection under normal white light and palpation of suspicious lesions, typically performed by dentists or physicians.

The main contribution of light-based methods, autofluoresence, confocal reflectance imaging, and fluorescence imaging is to highlight oral lesions and to assist the physicians to better locate the surgical margins. Most of the noninvasive detection techniques evaluated in this review showed great potential for screening and monitoring oral precancer and cancer. However, there is lack of sufficient data for the use of noninvasive techniques in detecting oral cancer at an early stage. Therefore, the studies are to be conducted in large sample of population to prove the effectiveness of light-based system and fluorescent methods to prove their effectiveness.

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