

Adjunctive Methods and Devices for Clinical Detection of Oral Squamous Cell Carcinoma

Hülya Çankaya^a/Pelin Güneri^a/Joel B. Epstein^b

Abstract: Oral squamous cell carcinoma (OSCC) is the most prevalent cancer of the head and neck with over 500,000 new cases every year worldwide. The stage of disease at diagnosis is associated with the 5-year survival rate. Unfortunately, approximately two-thirds of patients are diagnosed with advanced disease with local and regional or distant spread. Earlier detection of OSCC may be improved with the development of adjunctive techniques for clinical detection and diagnosis, which is expected to enhance the prognosis of the disease. This narrative review aims to provide an overview of adjuncts that are available for clinical use to assist in improving detection of potentially malignant epithelial lesions and early-stage OSCC.

Key words: cytology, diagnostic techniques, fluorescence, oral cancer, toluidine blue

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Squamous cell carcinoma (SCC) of the oral cavity is generally defined as 'oral cancer' and is the sixth most common malignancy in the world (Fedele, 2009; Trullenque-Eiksson et al, 2009; Mehrotra and Gupta, 2011). Despite recent advances in treatment modalities and improved cure rate, the 5-year survival varies widely by stage at the time of diagnosis (Seoane et al, 2006; Epstein et al, 2008c; Bagan et al, 2010). It ranges from 81.8% for patients diagnosed in localised stages to 52.1% for patients with regional lymph node involvement, and to 26.5% for patients with distant metastasis (Horner et al, 2009). Even though early-stage detection and diagnosis would lead to improved patient outcomes (Rana et al, 2012), the lack of public awareness about risk factors for oral cancer, few and nonspecific signs and symptoms as well as limited early detection by health-care providers are among the factors that may cause delayed diagnosis and more advanced disease stage at diagnosis (Thomson, 2002; Fedele, 2009; Scully and Petti, 2010; Cleveland et al, 2011).

Accurate diagnosis of any lesion can only be made via histological examination (Holmstrup et al, 2007). Despite this, the histologic diagnosis of dysplasia and SCC is subjective and both intra- and inter-rater variability is known (Pentenero et al, 2003; Fischer et al, 2004; Fischer et al, 2005). However, many oral squamous cell carcinomas (OSCCs) are preceded by visible changes in the oral mucosa, usually white (leukoplakia) and/or red patches (erythroplakia) (Fedele, 2009; Mehrotra and Gupta 2011) (Fig 1). The clinical presentations of the pioneer oral lesions include colour change, variations of the surface texture and integrity, al-

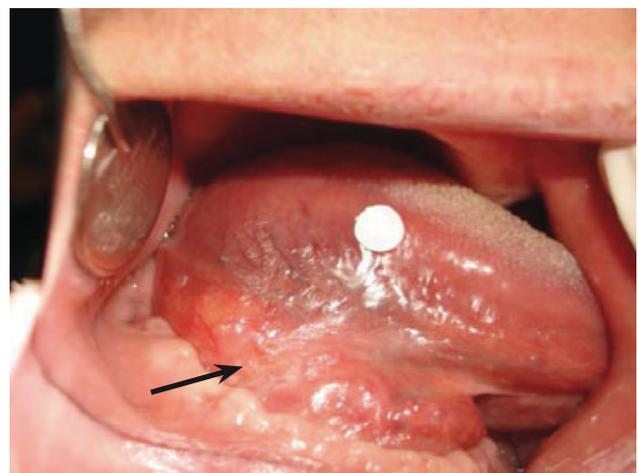


Fig 1 Chronic ulcerative lesion (arrow) with enrolled and firm borders that was diagnosed as OSCC. White dot is used for correction of light on the image.

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terations of the size and margins or mobility of adjacent structures (Epstein et al, 2008b,c). Although clinically normal-appearing oral mucosa may harbour malignant molecular transformations (Thomson, 2002), oral potentially malignant epithelial lesions (OPMELs) may signal the evolution of cancer (Neville and Day, 2002). Identification/monitoring of OPMELs facilitate clinical detection and treatment of early intraepithelial stages of oral carcinogenesis (mild, moderate or severe dysplasia and carcinoma in situ) before development of invasive OSCC (Fedele, 2009; Mehrotra and Gupta, 2011). Unfortunately, the clinical findings may not predict the histological findings (Epstein et al, 2012). Nevertheless, periodic clinical oral examination that includes evaluation of oral mucosa with visual inspection and palpation as well as thorough head and neck examination are imperative to detect abnormal oral mucosal transformations (Huber et al, 2004; Kerr et al, 2006; Farah and McCullough 2007; Epstein et al, 2008a). The 'index' of suspicion should be high and any identified lesion should be reviewed at a follow-up exam. If a lesion presents with features suggestive of irregular growth patterns and symptoms, histological confirmation is required. Unfortunately, visual identification of early lesions can be arduous even for experienced clinicians, since some precancerous lesions may appear clinically normal (Thomson, 2002) and normal tissues can sometimes exhibit benign changes (Schwarz et al, 2009). Furthermore, even histological evaluation is subjective, as described above (Fischer et al, 2004; Fischer et al, 2005).

Several adjunct detection/visualisation aids have been introduced to assist in the detection of early cancerous oral mucosal changes and to provide additional clinical information assessing the biological potential of clinically abnormal mucosal lesions (Epstein et al, 2007; Allegra et al, 2009; Fedele, 2009; Mehrotra, 2012). These products and devices include toluidine blue; the OralCDx BrushTest (OralCDx Laboratories; Suffern, NY, USA), an oral brush cytology test; ViziLite Plus (Zila Pharmaceuticals; Phoenix, AZ, USA), a direct tissue visualisation technique using acetic acid and a blue light source followed by toluidine blue; and the VELscope (LED Dental; White Rock, BC, Canada), a handheld device for direct visualisation of tissue fluorescence (Epstein et al, 2007; Mehrotra and Gupta, 2011).

Optical coherence tomography (OCT) is an imaging modality that is similar to ultrasound techniques, but the intensity of back-scattered light rather than sound waves is measured as a function

of depth in the tissue (Patil et al, 2008; Evans et al, 2009). Even though it lacks molecular specificity, it is a powerful volumetric imaging modality for visualising tissue microstructure with high volumetric resolution and has potential to provide real-time information that may assist the clinician in detection, accelerate the decision to biopsy and assist in identifying biopsy site selection and margin determination (Patil et al, 2008).

This paper aims to review current evidence regarding available clinical diagnostic aids for detection of potentially malignant mucosal lesions and promote early detection of OSCC. The findings on examination and with adjunct use are compared to the histologic findings which serve as the gold standard in diagnosis.

EXFOLIATIVE CYTOLOGY / ORAL BRUSH CYTOLOGY

Oral exfoliative cytology has been used since the 1950s to obtain epithelial cells; modification of collection with a bristle brush has been shown to include basal epithelial cells and allow examination of cell morphology under a light microscope (Huang et al, 1999; Bloching et al, 2000). This represents the oral application of approaches used for cervical cancer detection and diagnosis (PAP smear). It is promoted as a rapid, inexpensive and well-tolerated method which may help evaluate the need for scalpel biopsies in clinically benign-appearing lesions (Silverman, 1988; Huang et al, 1999; Sciubba, 1999; Walling et al, 2003; Trullenque-Eiksson et al, 2009; Mehrotra et al, 2010). In 1999, the OralCDx Brush Test system (oral brush cytology) was introduced as a potential oral cancer case-finding device (Fedele, 2009). This test was specifically designed to investigate mucosal abnormalities that would otherwise not be subjected to biopsy because of low-risk clinical features (Sciubba, 1999; Eisen, 2002; Frist, 2003; Eisen and Frist, 2005; Fedele, 2009). In this method, no topical or local anaesthesia is required. A specially designed brush is utilised to obtain a transepithelial sample of cells from a mucosal lesion with representation of the superficial, intermediate and parabasal/basal layers of the epithelium (Sciubba, 1999; Eisen, 2002; Frist, 2003; Eisen and Frist, 2005; Fedele, 2009). The brush is placed on the lesion surface and is rotated 5 to 10 times until it produces reddening or hemorrhagic spots which suggests that the basal layer of the epithelium is

reached. The cell material obtained is transferred to the slide and fixed (Sciubba, 1999; Ujaoney et al, 2012). The initial histological evaluation is performed via a computer programme based on the 'image recognition process', comparable to current PAP smear evaluation for cervical lesions. When cellular morphology is suspicious for epithelial dysplasia or carcinoma, or when abnormal epithelial changes are of uncertain diagnostic significance, the results are reported as 'positive' or 'atypical', respectively. In this case, the clinician must follow up with a scalpel biopsy of the lesion (Fedele, 2009; Mehrotra and Gupta, 2011). When no abnormalities are observed, the results are reported as negative. However, oral brush cytology does not provide a definitive diagnosis and surgical biopsy remains the only diagnostic method (Fedele, 2009).

Several studies have assessed the sensitivity and specificity of brush cytology in detecting dysplasia or OSCC (Sciubba, 1999; Eisen, 2002; Frist, 2003; Potter et al, 2003; Rick, 2003; Poate et al, 2004; Scheifele et al, 2004; Eisen and Frist, 2005; Fedele, 2009), but the results are controversial (Rethman et al, 2010). While a number of reports have shown the value of brush cytology (Cowpe et al, 1988; Silverman, 1988; Huang et al, 1999; Kujan et al, 2006; Gupta et al, 2007; Mehrotra and Gupta, 2011), others reported large numbers of false positive and false negative results obtained with this method (Epstein et al, 1997; Onofre et al, 2001; Ram and Siar, 2005; Gandolfo et al, 2006), ranging from 30%–84% (Hodgson et al, 2002) to 63% for dysplastic lesions (Oral Cancer Foundation, 2011). Nevertheless, the design of the studies may influence the sensitivity, specificity and the positive predictive values of the brush biopsy technique. The population sample can affect the false positive and false negative results and alter the positive predictive value of brush biopsy according to the prevalence of the disease in a study sample. Further, in many studies, scalpel biopsy was performed after brush biopsy of lesions with high-risk clinical features, but this procedure was not carried out for clinically benign-appearing lesions (Fedele, 2009), even though the histological evaluation of the latter is required to establish the accuracy of the brush biopsy in the clinical context (Potter et al, 2003; Rick, 2003; Fedele, 2009). Additionally, in order to make valid comparisons between brush sampling vs scalpel biopsy, only studies comparing the results of both biopsies performed at the same time and from the same portion of the suspicious lesion should be considered valid (Mehrotra and Gupta,

2011; Mehrotra et al, 2011). Otherwise, the biopsy and final diagnoses of the lesions would vary over time due to the changes within the course of the lesion (Holmstrup et al, 2007; Mehrotra et al, 2011). Mehrotra et al (2011) performed such a study where immediate scalpel biopsies were obtained after brush biopsies from oral lesions, reporting 96.3% sensitivity and 100% specificity for dysplasia or carcinoma. Potential limitations of this method have been reported, e.g. transepithelial collections may not be possible in necrosis and/or mucosal infection, which are frequently observed with carcinomas (Trullenque-Eiksson et al, 2009; Rethman et al, 2010). Additionally, mucosal sites and lesions with a high degree of keratinisation may prevent collection of enough basal cells samples in leukoplakia, and inflammatory conditions may frequently lead to atypical results (Trullenque-Eiksson et al, 2009; Rethman et al, 2010; Mehrotra, 2012).

The potential for dysplasia to resolve or progress presents a challenge in diagnosis. The differentiation between atypia associated with inflammation as compared to neoplastic change creates an additional cause of variable diagnosis and progression. As the definition of histological dysplasia is largely a subjective expression of the examiner, variability in diagnosis of PMEL has been documented (Fischer et al, 2004; Fischer et al, 2005). It has been shown that the presence or absence of dysplasia appeared to have no influence on the course/transformation of potentially malignant lesions (Holmstrup et al, 2007), which should be followed by observations every 3 to 6 months in order to detect the malignant changes early on (Holmstrup et al, 2007; Rethman et al, 2010). Exfoliative cytology may be propitious in patients with potentially malignant lesions (Rethman et al, 2010) or multiple lesions throughout the oral cavity that require multiple incisional biopsies (Rethman et al, 2010; Mehrotra, 2012), following oral cancer therapy where mucosal changes occur due to therapy and in non-compliant patients who would not return for a follow-up exam or accept an immediate biopsy or referral for evaluation and possible biopsy by an experienced provider (Rethman et al, 2010). It must be remembered that cytology is not a diagnostic test and cannot be relied upon for diagnosis, and if lesions progress over time, a new biopsy is needed. Indeed, applying molecular measures to exfoliated cells has been and continues to be studied in order to improve the utility of cell collections.

TOLUIDINE BLUE STAINING

Toluidine blue (TB), also known as tolonium chloride, is a metachromatic vital dye that may bind preferentially to tissues undergoing rapid cell division (such as inflammatory, regenerative and neoplastic tissue) and to sites of DNA change associated with PMELs (Allegra et al, 2009). It has been used for more than 40 years as a vital stain to aid in detection of potentially malignant abnormalities of the uterine cervix and the oral cavity (Mashberg, 1983; Rosenberg and Cretin, 1989; Onofre et al, 2001). The method of application of TB may be either as a mouthrinse or topical. A second follow-up visit approximately two weeks after the first application is recommended, because traumatic and inflammatory mucosal changes and ulcerations also have high cellular metabolic rates and they may lead to false-positive findings (Patton et al, 2008; Cancela-Rodríguez et al, 2011).

TB binding results in the royal blue staining of abnormal tissue in contrast to adjacent normal mucosa (Gandolfo, 2006; Patton et al, 2008) (Fig 2). It has been linked with loss of tumour suppressor gene (TSG) loci on specific chromosomes that predict progression to cancer. The relevance of TB has been reported for identification of PMELs and early diagnosis of OSCC (Mashberg, 1980; Mashberg, 1983; Epstein et al, 2003a; Zhang et al, 2005; Gandolfo, 2006; Patton et al, 2008), assessing margins of oral potentially malignant lesions and SCC of the lesions before biopsy (Portugal et al, 1996; Kerawala et al, 2000; Missmann et al, 2006; Siddiqui et al, 2006; Driemel et al, 2007), assisting in biopsy site selection (Missmann et al,



Fig 2 Royal blue staining of the lesion shown in Fig 1, indicating binding of tolonium chloride of abnormal tissue in contrast to adjacent normal mucosa. White dot is used for correction of light on the image.

2006; Siddiqui et al, 2006; Driemel et al, 2007) and follow-up of patients with previous oral cancer (Epstein et al, 2003a).

Even though it is recommended that only dark royal-blue staining should be regarded positive and no/pale blue staining should be considered negative (Gandolfo et al, 2006), some studies that investigated the efficacy of TB staining considered any uptake of blue dye positive, while others classified partial staining either as positive or negative, or assigned to another category (Patton et al, 2008; Ujaoney et al, 2012). This difference in the clinical interpretation of variations of the intensity of stain (dark or pale royal blue) of the area (Mashberg, 1980; Mashberg, 1983; Silverman et al, 1984; Portugal et al, 1996; Warnakulasuriya and Johnson 1996; Martin et al, 1998; Kerawala et al, 2000; Onofre et al, 2001; Epstein et al, 2003a; Gandolfo et al, 2006) widened the range of the sensitivity/specificity and positive predictive value (PPV)/negative predictive value (NPV) values of TB staining: sensitivity changed between 38%–98%, specificity between 9%–93%, PPV between 33%–93% and NPV between 22%–92% (Mashberg, 1980; Mashberg, 1983; Silverman et al, 1984; Epstein et al, 1992; Warnakulasuriya and Johnson 1996; Epstein et al, 1997; Onofre et al, 2001; Epstein et al, 2003a,b; Ram and Siar, 2005; Zhang et al, 2005; Patton et al, 2008). The sensitivity and specificity of TB was reported to be higher for malignant lesions, but it was less sensitive for potentially malignant lesions (Gupta et al, 2007). Additionally, it has been reported that TB has higher sensitivity in identifying suspicious lesions when their clinical examination gives negative results (Allegra et al, 2009).

A high rate of false-positive results of TB staining has been related to the high cellular metabolic rate of other tissues which may retain TB and appear dark blue (Cancela-Rodríguez et al, 2011). On the other hand, TB may be positive in the face of abnormal molecular changes present in cancer or in predicting the progression of PMEL to cancer as well (Guo et al, 2001; Epstein et al, 2003a; Zhang et al, 2005). TB has been recommended for use in high-risk populations, such as those encountered by referral centres and expert examiners (Epstein 2008a; Patton et al, 2008; Rethman et al, 2010; Ujaoney et al, 2012), although limited guidance is provided for general populations and providers because the number of studies conducted in these settings is rare and there is increased risk of false-positive and false-negative results in low risk (low

prevalence) settings. Actually, molecular abnormalities may be present at margins of lesions and at oral sites distant from the clinical lesion which may be considered clinically and even histologically benign (Partridge et al, 2000; Epstein et al, 2002; Hodgson et al, 2002; Braakhuis et al, 2003; Patton et al, 2008). Considering that positive TB staining presents lesions with loss of heterozygosity and may precede histological transformation (Guo et al, 2001; Epstein et al, 2003a; Zhang et al, 2005) due to binding to sites of molecular change, some previously suggested 'false positive' TB results based upon histomorphology may actually represent molecularly 'true positive' lesions with risk of progression to OSCC (Guo et al, 2001). Therefore, the 'high false positivity of TB' reported in some studies may need to be reconsidered (Güneri and Epstein, 2010).

When applied by experienced clinicians, TB staining as an adjunct may prove useful in the evaluation of oral mucosal lesions and also in the surveillance of high-risk individuals, such as patients at risk for a second primary lesion. This is recommended in various systematic reviews (Rosenberg and Cretin, 1989; Patton et al, 2008; Rethman et al, 2010).

LIGHT-BASED DETECTION SYSTEMS

The development of oral neoplasia is associated with abnormal metabolic and structural changes in tissue optical properties, such as the discrepancies in the fluorophore concentrations, fluorescent collagen crosslinks within the stroma, tissue scattering, hemoglobin absorption and tissue thickness (Pavlova et al, 2008; Schwarz et al, 2009). Therefore, fluorescence diagnostics have been developed to detect the pathological changes in tissues (Sieroń et al, 2008) and light-based detection systems are based on the assumption that suspicious mucosal tissues might reveal different absorbance and reflectance profiles when exposed to various forms of light or energy (Swinson et al, 2006; Patton et al, 2008; Leston and Dios, 2010). Supporting this concept, some authors report progressive reduction in blue-green intensity of light in dysplastic and cancerous tissues (de Veld et al, 2004; Poh et al, 2006; Swinson et al, 2006; Poh et al, 2007; Schwarz et al, 2009). Tissue fluorescence loss has been associated with loss of heterozygosity in oral mucosa (Poh et al, 2006; Poh et al, 2007). However, using this method to distinguish dysplastic lesions from malignant ones (Schwarz et al, 2009) or

inflammatory areas from dysplasia remains challenging (Pavlova et al, 2008), probably due to variations in photosensitive compound contents (Sieroń et al, 2008; McNamara et al, 2012).

ViziLite system

Several devices that utilise light for assisting clinical oral cancer diagnosis have been introduced on the dental market. Among these, ViziLite system (Zila Pharmaceuticals; Phoenix, AZ, USA) became the first system cleared by the FDA Devices Branch to improve the visualisation of early cancer lesions in head and neck examinations using reflectance properties of the tissues. The kit consists of a 1% acetic acid solution, a capsule which emits light when activated and Tblue acetic acid swabs (Ram and Siar, 2005; Ujaoney et al, 2012). For light activation, the capsule is bent, breaking the inner glass vial so that the chemical products react and produce a bluish-white light with a wavelength of 430–580 nm that lasts for around 10 minutes (Ram and Siar, 2005). The ambient light is dimmed and a diffuse bluish-white chemiluminescent light is applied. Normal cells absorb the light and have a bluish colour, whereas the light is reflected by abnormal cells with a higher nucleus-to-cytoplasm ratio and by epithelium with excessive keratinisation, hyperpara-keratinisation and/or significant inflammatory infiltrate, leading to an aceto-white appearance with brighter, marked and clinically distinguishable borders (Huber et al, 2004; Ram and Siar, 2005; Epstein et al, 2006; Kerr et al, 2006; Farah and McCullough, 2007; Oh and Laskin, 2007; Schwarz et al, 2010). The efficacy of the ViziLite system to enhance the identification of mucosal abnormalities has been investigated (Ram and Siar, 2005; Epstein et al, 2006; Kerr et al, 2006; Farah and McCullough, 2007; Oh and Laskin, 2007; Ujaoney et al, 2012), but varying results have been reported. Some authors report an increased ability of clinicians to detect mainly white and white-red oral lesions with utilisation of ViziLite (Huber et al, 2004; Epstein et al, 2006; Epstein et al, 2008a; Trullenque-Eiksson et al, 2009) due to the increased brightness and sharpness of margins (Epstein et al, 2008b; Epstein and Güneri, 2009; Leston and Dios, 2010) (Fig 3). On the other hand, its low potential for discrimination between malignant, benign and inflammatory oral lesions (Farah and McCullough 2007; Oh and Laskin, 2007; Patton et al, 2008; Leston and Dios, 2010; Ujaoney et al,



Fig 3 Chemiluminescent examination of the lesion shown in Fig 1 with ViziLite, revealing increased brightness and sharpness of margins.

2012) as well as low specificity and high rate of false positives – which could necessitate unnecessary biopsies – has been discussed in some papers (Ram and Siar, 2005; Patton et al, 2008; Trullenque-Eiksson et al, 2009). In order to overcome this drawback and to reduce the number of false positives without increasing the rate of false negatives, combination of ViziLite with toluidine blue has been proposed (ViziLite Plus) (Epstein et al, 2008a). Epstein et al (2008b) stated that enhanced sharpness and brightness of oral white lesions may improve visualisation with ViziLite Plus: all lesions with dysplasia and carcinoma in a high-risk patient population were identified and fewer false-negative findings were reported, suggesting that TB may assist in achieving the accurate diagnosis of approximately one-half the number of biopsies of other mucosal lesions and all PMELs/OSCCs (Epstein et al, 2008b).

Microlux Diagnostic Light

Microlux Diagnostic Light (Microlux DL, AdDent; Danbury, CT, USA) is another device that uses refractive light technology in the detection of precancerous oral mucosal abnormalities, promoted for use as an adjunct to conventional oral mucosal exams (AdDent, 2010); however, only one study with this device was identified. McIntosh et al (2009) reported that Microlux DL enhanced the visibility of oral white lesions, but could not discriminate their inflammatory, benign or malignant nature. OraSoptic DK (OraSoptic, Kerr; Middleton, WI, USA) is a device similar to Microlux, producing light of

similar wavelength to ViziLite. Unfortunately, the lack of published studies related to the efficacy of these instruments does not provide sufficient evidence to develop recommendations for use (Patton et al, 2008; Rethman et al, 2010).

Visually Enhanced Lesion Scope (VELscope)

The Visually Enhanced Lesion Scope (VELscope) (LED Dental) uses the fluorescence properties of oral mucosa. It is not a diagnostic device, but rather is marketed as a tool that facilitates the discovery of mucosal abnormalities before they become visible under incandescent light when used in conjunction with the conventional oral and head and neck exam (Poh et al, 2006; Poh et al, 2007; Trullenque-Eiksson et al, 2009; Poh et al, 2011; Scheer et al, 2011; Farah et al, 2012). The VELscope handpiece emits a blue light, which excites natural fluorophores through the surface of the epithelium to the basement membrane and into the stroma, causing it to fluoresce (Fig 4) (Poh et al, 2006; Poh et al, 2007; VELscope, 2010; Poh et al, 2011; Scheer et al, 2011; Farah et al, 2012). It is promoted to assess fluorescence changes in early pathological phases before becoming clinically evident (Trullenque-Eiksson et al, 2009).

The studies investigating the clinical value of the VELscope are limited, but ongoing. Huff et al (2009) revealed increased detectability of oral mucosal abnormalities when incandescent light examination was used in conjunction with the VELscope. This finding was supported in another study where the sensitivity of identification of malignant and dysplastic areas with the VELscope was 100% and the specificity was 80.8%. However, the discriminatory ability between malignant and other conditions remains uncertain because of its low positive predictive value (54.5%) (Scheer et al, 2011). Awan et al (2001) reported 84.1% sensitivity and 15.3% specificity of autofluorescence for the detection of a dysplastic lesion, and pointed out that even though the VELscope was useful in detecting oral leukoplakia and erythroplakia, it was unable to discriminate high-risk malignant or potentially malignant lesions from low-risk ones. In a recent study, Rana et al (2012) showed that the use of the VELscope led to higher sensitivity (100% instead of 17%) but lower specificity (74% instead of 97%) when compared to those of clinical examination alone. In contrast, Mehrotra et al (2010) showed that neither ViziLite nor VELscope was beneficial in identifying dysplasia

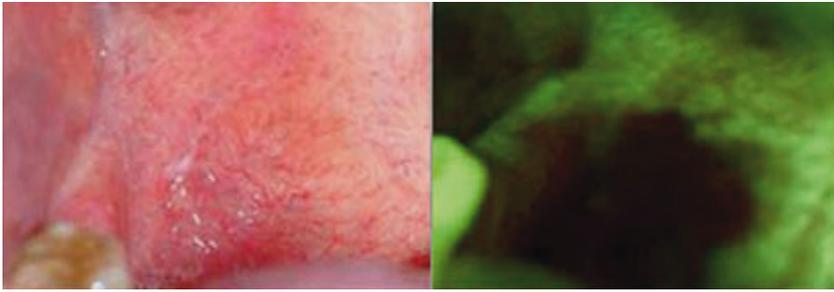


Fig 4 Utilisation of VELscope for the discovery of mucosal abnormalities (available at <http://www.velscope.com>).

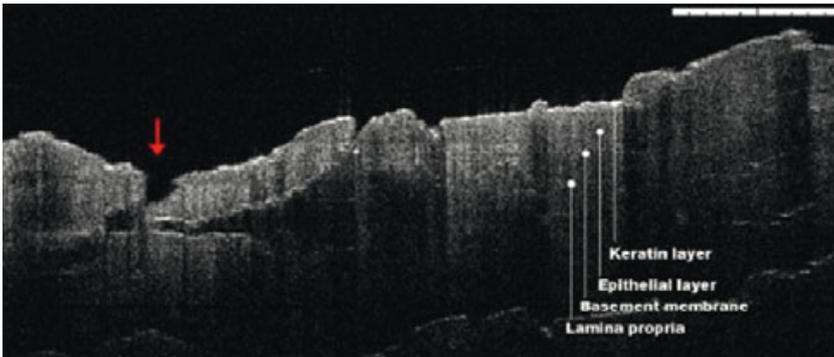


Fig 5 Optical coherence tomography image of a lesion, providing millimeter penetration with micrometer-scale axial and lateral resolution (Jerjes et al, 2010).

or oral cancer in high risk populations in India, when used as an auxiliary method with clinical examination. VELscope showed high false-positive rates when used to screen routinely for oral cancer; thus, such devices were recommended to be reserved for use in opportunistic screening programmes or in cancer referral clinics (Balevi, 2011). It is reported that common inflammatory conditions including traumatic ulceration, benign migratory glossitis, inflammatory papillary hyperplasia, chronic mucositis and areas rich in lymphoid tissue or melanin pigmentation may cause loss of visual fluorescence (McNamara et al, 2012). Moreover, the attached gingiva and tonsillar pillars, as well as mucosa with prominent physiological pigmentation, reduce the visual fluorescence of oral mucosa, which affects the utility of direct visual fluorescent examination (McNamara et al, 2012). In short, it is stated that rather than determining whether a lesion is precancerous or cancerous, these oral cancer screening lights should only be used to help identify lesions that may have been overlooked with a conventional oral examination (Mehrotra and Gupta, 2011). Thus, it appears that use of VELscope in detection/screening has limited data supporting its use in the general population, due to false positive and negative results. This is largely based on its limited ability to distinguish inflammatory from dysplastic lesions. The best support in the literature for the use of fluorescence visualisation is in margin delineation in already diagnosed malignant lesions.

Identafi 3000

In addition to the above mentioned devices, another instrument that uses both light reflectance or fluorescence properties of the tissues has been introduced (Identafi 3000, Trimira; Houston, TX, USA). It employs a multi-spectral method with three distinct colour wavelengths (white, violet, amber) to distinguish lesion morphology and vasculature (Trimira, 2012). Nevertheless, determining the actual value of these devices in clinical practice requires studies on larger patient samples, using more detailed histological and molecular mapping of the area of interest, evaluating the contributing/affecting factors of the optical properties of the lesion and examining the concordance with clinical findings (Westra and Sidransky, 2006; Huff et al, 2009; Balevi, 2011).

Optical coherence tomography (OCT)

Optical coherence tomography (OCT) is a light analogue of ultrasound with much higher resolution that appears to hold promise in the field of clinical diagnosis and monitoring of dysplasia and cancer. It is a non-invasive, tomographic imaging modality that provides millimeter penetration with micrometer-scale resolution (Evans et al, 2009; Jerjes et al, 2010). In this system, light is broken into two arms: a reference arm (a mirror) and a sample arm (used

to scan the sample of tissue). Combination of the light from both arms produces an interference pattern; then, subsurface reflections are used to build a cross-sectional architectural image of tissue. This light pattern is picked up by the detector and converted into a representative image pixel. Areas of the tissue sample that reflect light will create a larger signal, i.e. a higher resolution image (Jerjes et al, 2010) (Fig 5). The applicability of this method in oral and laryngeal lesion diagnosis has been investigated (Wong et al, 2005; Armstrong et al, 2006; Ridgway et al, 2006; Wilder-Smith et al, 2009) with promising results regarding the use of light in clinical practice. The primary limitation of OCT is that the images are reflectivity maps of sample morphology (Patil et al, 2008).

Raman spectroscopy

Raman spectroscopy is another light-based method that involves the inelastic scattering of photons by interaction with molecular bonds of the materials and potentially provides additional molecular information (Evans et al, 2009). The principle is that illumination of a material by monochromatic light at an arbitrary wavelength leads to scattering of a fraction of the photons with a frequency shift that is related to the vibrational or rotational states of the molecular bonds within that material. Thus, it provides *in vitro/in vivo* molecular level information and is therefore particularly appealing in biomedicine (Evans et al, 2009). Recent reports supported the efficacy of Raman spectroscopic approaches in oral-cancer applications and showed that Raman spectroscopy had high sensitivity in detecting subtle oral mucosal changes (Sahu et al, 2012) as well as the ability to objectively discriminate potentially malignant conditions (Singh et al, 2012).

CONCLUSION

Adjunct devices and methods are employed either to increase the visibility of oral mucosal lesions or to provide non-invasive real-time data regarding the nature of the suspicious mucosal lesions. Unfortunately, their efficacy in clinical settings is inconsistent. The results of the studies investigating their efficacy vary according to the settings of the study and the sample population; that is, in a population of high-risk individuals, the adjunct devices/modal-

ities are expected to have better utility than in a general population. Furthermore, adjuncts have been studied primarily in referral clinic settings or cancer centres by experienced/expert examiners (Gupta et al, 2007; Rethman et al, 2010). Much less data is available on the adjuncts in general clinic settings, and therefore guidance for use is not provided.

PMEL and SCC are uncommon lesions and the differentiation between common inflammatory lesions is challenging. Moreover, in the highest risk patients who have had prior SCC, treatment-related tissue changes complicate the clinical assessment. The best evidence supports use of toluidine blue in the high risk setting, and toluidine blue and VELscope in margin delineation of identified lesions. The impact of false-positive results leads to potentially more invasive diagnosis and treatment, increasing cost of care and patient anxiety. False-negative findings carry a more potentially significant impact by delaying diagnosis. Many studies describe diagnoses of inflammatory lesions as false positive when the goal was to detect malignant disease. However, definitive diagnosis of a lesion whether inflammatory or potentially malignant is clinically useful, and may not be considered 'false positive', which challenges the interpretation of clinical studies. A comprehensive interview with the patient to obtain the medical-dental history and a thorough clinical, extraoral head and neck examination as well as an intraoral examination remain as the initial components of an accurate clinical diagnosis. Nonetheless, the final diagnosis is made only with histological evaluation of the lesion.

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