VELscope Vx Design Validation Report
Clinical Studies

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1 Objective

The objective of this report is to present clinical experience data related to LED Dental Inc.’s VELscope® Vx system to validate the device relative to the indications for use. The key questions:

- Is there sufficient clinical evidence to support that the VELscope Vx device performs in accordance with its Indications for Use?
- Does the reported clinical experience with the VELscope Vx device support that the risk associated with the use of the device is acceptable as stated in the VELscope Vx Risk Management File?

It is also intended to satisfy the requirements of Annex 10 of the European Medical Device Directive and follows the guidelines as per MEDDEV 2.7.1Rev 3, “Clinical Evaluation: A Guide for Manufacturers and Notified Bodies”.

This report was compiled by Dr. David Morgan, Chief Science Officer of LED Dental Inc. Dr. Morgan has a PhD in physics from the University of British Columbia (UBC) and has held post-doctoral research fellowships at the University of Cambridge in the UK, UBC and the BC Cancer Research Centre in Vancouver, BC. He has had 15 years of experience in the research and development field of medical devices. In particular, he has spent the majority of this time directly in the field relating to the use of fluorescence as an aid in the detection of disease in human tissues. His activities have included fundamental research, product development, quality systems, clinical affairs and regulatory affairs.

2 Scope

This document applies to the latest generation VELscope Vx system. Since the fundamental principle of operation is the same as the earlier VELscope systems, the data in this report includes a clinical comparison between these early generation VELscopes and the latest VELscope Vx, as well as clinical data validating the use of the VELscope.

With the exception of the references listed below, all published literature in English known to the author of this document reporting clinical data using the VELscope or VELscope Vx system was included in this report. In addition, 2 literature searches were performed on the PubMed database: one with the key words ‘autofluorescence’ and ‘VELscope’ and the second with ‘fluorescence’ and ‘VELscope’. This was to find any relevant references that may have been missed.
There were 5 references that were found in the literature search and are not included in this report. They are listed below along with a brief justification for their omission from this report:

  - This itself was a review of other published clinical work and so did not include any new clinical data.

  - The focus of this paper was on patient education and acceptance and did not include any new clinical data relevant to performance in accordance with the Indications for Use.

- “Evidence-based decision making: should the general dentist adopt the use of the VELscope for routine screening for oral cancer?” Balevi, J Can Dent Assoc. 2007 Sep;73(7):603-6.
  - This was a review and an editorial piece and did not include any new clinical data.

  - This was a case report on a single lesion, a squamous papilloma, well understood to have a definite response under fluorescence examination. No new insight was gained from this work other than highlighting that the VELscope can be useful to detect lesions unrelated to dysplasia or cancer.

3 Context for Evaluation of Clinical Data – Clinical Claims & Intended Use

It is very important when considering clinical design validation data to evaluate them within the context of the clinical claims and intended use of the device. The Indications for Use of the VELscope Vx device are identical to the predecessor VELscope models and are reproduced here for completeness:

*The VELscope® Vx System is intended to be used by a dentist or health-care provider as an adjunct to traditional oral examination by incandescent light to enhance the visualization of oral mucosal abnormalities that may not be apparent or visible to the naked eye, such as oral cancer or pre-malignant dysplasia.*
The VELscope® Vx System is further intended to be used by a surgeon to help identify diseased tissue around a clinically apparent lesion and thus aid in determining the appropriate margin for surgical excision.

To understand the correct clinical use of the VELscope, there are a few key points to remember. First, the device is intended as an aid to the clinician to complement a conventional white light exam (whether it be a general oral cavity examination or examination of a particular lesion) to detect abnormal tissue that might have otherwise been overlooked. It is not a device that is intended in and of itself to discriminate between healthy tissue and diseased tissue. Nor is it in any sense claimed to be specific to precancerous or cancerous tissue; these are mentioned prominently in the Indications for Use but only as examples (note the use of the wording “…such as…”) of some of the more significant lesions warranting attention by clinicians. There are many other examples of lesions that also require varying degrees of follow-up by clinicians that are completely unrelated to precancer or cancer. In both the general examination and surgical contexts, the device is best thought of as an adjunctive tool used in combination with the conventional white light examination – assisting in the detection of abnormal areas potentially warranting further investigation and then contextualization of that information within the larger clinical picture. It is this whole process that determines the course of action for the clinician. Although terms such as “positive”, “negative”, “sensitivity” and “specificity” are somewhat common in the academic literature, it is important to understand that they are not well defined for the sense in which the device is intended to be used. To put it in plain language, how to act upon the observation with the device of a particular area of loss of fluorescence varies significantly depending on many other factors, some of which are independent of the use of the device. This should not be considered surprising since this is exactly the way traditional observations of oral mucosa made under conventional white lighting are treated.

To summarize, the VELscope is not a diagnostic device. It is part of the diagnostic process for the evaluation oral lesions in the same way that observation of the mucosa under traditional illumination is part of this process as well: for the purpose of identifying areas of possibly unhealthy tissue that warrant further follow-up. To reiterate, this possibly unhealthy tissue may turn out to be dysplastic or cancerous but it is much more likely that it be a result of a myriad of other possible causes (e.g. trauma, infection, etc.). See below for a block diagram representation of the oral mucosal diagnostic process.
Note that the addition of the VELscope examination to the diagnostic process enables the possibility of enhanced lesion discovery. Also, this diagram is a valuable reminder of the other very important elements of the diagnostic process: patient interview and questionnaires (health history, risk factors, medications etc…) and most importantly clinical judgment as it pertains to the establishment of a differential diagnosis and identification of next steps. The patient information and results of the VELscope examination (like the traditional head and neck exam) provide information to the clinician which is the input for the application of clinical judgment. None of these other steps (including the VELscope examination) in any way diminish the importance, or changes the role, of surgical biopsy which remains the gold standard for histopathology-based diagnosis.
Therefore, the significance of the clinical data as it pertains to validation of device design must be considered in the context of the Indications for Use – i.e. does the data validate that the VELscope Vx enhances “the visualization of oral mucosal abnormalities” etc…? The VELscope Vx and the earlier VELscope device are by their nature qualitative rather than quantitative tools.

4 Equivalence of VELscope and VELscope Vx systems

The clinical performance of the latest generation VELscope Vx system is equivalent to the earlier generation VELscope system. The reasoning is provided below. This is essentially the substantial equivalence argument that was included in the VELscope Vx 510(k) application to the US FDA. This 510(k) was cleared by the FDA in 2010.

- The VELscope Vx System in this submission has identical Indications for Use as the predicate device.
- Although changes have been made with respect to the originally cleared VELscope System to integrate the light source into the Handpiece and to allow cordless operation by means of a rechargeable battery, the theory of operation, underlying mechanisms of action and key performance specifications, are unchanged.

The key mechanism of action relevant to the intended use of the device is the generation of natural tissue fluorescence from the oral mucosa. The character of the fluorescence light generated and observed by the user is primarily dependent on:

- The wavelength of the excitation light.
  - This is dictated by the selected bandwidth chosen for the excitation filtering. The excitation filter used for the VELscope Vx system has identical specifications to the one used in the predicate device. The excitation bandwidths for both systems are thus identical: 400 – 460nm. Both the blue LED’s in the VELscope Vx system and the metal halide lamp in the earlier VELscope system (predicate device) deliver substantial light across this wavelength range, and the identical filtering leads to an excitation band of 400-460nm in both cases.
- The wavelengths of the emitted fluorescence light, viewable by the user.
  - This dictated by the specifications of the filters in the viewing optical path which again are unchanged from the predicate device. The bandwidth of the fluorescence viewed by the user on both systems is >475nm with a stop band from 560 – 620nm.
Thus the theory of operation of the VELscope Vx device and the mechanisms underlying the tissue fluorescence visualized with it are identical to the original VELscope system. Correspondingly, the supportive references cited in that application are just as applicable to the VELscope Vx system.

The character of the fluorescence generated by the tissue is also dependent to a lesser extent on the absolute power of the excitation light in the 400-460nm band. The absolute power of the blue excitation light is directly related to the brightness of the resulting fluorescence. This is a user convenience issue because brighter fluorescence images are somewhat easier to photo-document with a camera. The VELscope Vx was designed to be comparable with the originally cleared VELscope in this regard as well:

- Typical optical excitation power inside a 4cm diameter spot at 10cm from the front face of the VELscope Handpiece for the original VELscope is ~1.0 W.
- Typical optical excitation power inside a 4cm diameter spot at 10cm from the front face of the VELscope Handpiece for the VELscope Vx is also ~ 1.0 W.

Apart from improved ergonomics, the cordless operation by means of a rechargeable battery has no effect on the essential performance of the VELscope Vx system as compared to the original VELscope system.

The only difference that might have the potential to affect clinical performance is that the spectral shape of the blue excitation light within the 400-460nm excitation band for the VELscope Vx system is slightly different because the combination of the 16 blue LED’s leads to a slightly different distribution of light than the mercury/metal halide lamp used in the original VELscope.

To investigate the possible effect of this minor change to the wavelength distribution inside the excitation band, a VELscope Vx device and a VELscope device were used to photograph a variety of lesions at the University of Washington (see below) and as can be seen from the photographs, no significant differences were observed.

**Clinical Photographs from the University of Washington**

Clinical photographs were taken of a variety of oral mucosal lesions from patients referred to oral medicine and oral dysplasia clinics at the University of Washington. Conventional (white light) as well as fluorescence photographs using both the predicate VELscope and the VELscope Vx were acquired. No adverse events or complications were reported. A comparison of the original VELscope and VELscope Vx images supports the equivalence of the VELscope Vx with the original VELscope.
Clinical Erythema
Palatal Pigmentation
Clinical

VELscope

VELscope Vx

Fibrotic Trauma
Clinical

Trauma,
Buccal Mucosa

VELscope

VELscope Vx
Dysplasia,
Buccal Mucosa

Clinical

VELscope

VELscope Vx
To gain some additional perspective on the types of changes to the qualitative appearance of abnormal or diseased tissue one might expect, see the figure below which is reproduced from Roblyer et al (Cancer Prev Res 2009;2(5) May 2009).

![Autofluorescence and white light images of the buccal mucosa of a typical study patient. A. White light image showing regions of interest of histopathologically confirmed normal tissue and invasive carcinoma. B. Fluorescence image at 365 nm excitation. C. Fluorescence image at 405 nm excitation. D. Fluorescence image at 450 nm excitation.](image)

As can be seen from the fluorescence photographs B, C and D above, the qualitative fluorescence appearance of the tissue is comparable for all three. These images were obtained with significantly different excitation wavelengths: 365nm (UV), 405nm and 450nm. All three can be said to offer together with their white light counterpart enhanced visualization of this cancerous lesion. Note that the differences in excitation wavelength seen here are much more profound than the discussed minor spectral differences between the original VELscope and the VELscope Vx in the excitation band 400-460nm. This is another argument that in addition to the pictorial evidence provided from the University of Washington
comparison that justifies the consideration of the many clinical studies conducted on the original VELscope as valid clinical design validation data for the VELscope Vx.

In conclusion, given the above demonstrated equivalence of the VELscope and VELscope Vx systems as it relates to essential clinical performance – performing as per the Indications for Use as a visualization adjunct, it is reasonable and correct to cite clinical studies performed with the original VELscope system as supportive clinical data for the VELscope Vx system.

5 Clinical Data Supporting the utility of VELscope & VELscope Vx (chronological order)


In this study reported by Lane et al, 50 biopsy samples were acquired from 44 patients (with a history of biopsy confirmed oral dysplasia or squamous cell carcinoma [SCC]):

- None of the 6 biopsy samples with a histological diagnosis of normal showed fluorescence visualization loss (FVL).
- 91% of high-grade pre-invasive lesions (severe dysplasia and carcinoma in situ [CIS]) showed FVL.
- 100% of invasive SCC showed FVL.

See Appendix A for a copy of this manuscript.


The authors report in this article 4 representative cases from their experience with the use of VELscope system during the Oral Dysplasia Clinic conducted by the department of Oral Medicine at the University of Washington Medical Center’s Head and Neck Cancer Clinic.

In all 4 cases, oral lesions that would have been missed or disregarded if assessed only through the conventional white light and palpation exam were carefully examined with the aid of the VELscope system. The device revealed/reinforced the presence of such lesions, which were subsequently confirmed through biopsy and histopathology to be either cancerous of pre-cancerous.

According to the authors, “[the VELscope system] has resulted in diagnoses of malignant disease or very aggressive dysplasia in a number of cases that would
have otherwise been judged clinically as negative for dangerous change.”

See Appendix B for a copy of this manuscript.


Poh et al reported the use of the VELscope device in the operating room to directly visualize field changes surrounding cancerous lesions in the oral mucosa that were not clinically apparent (i.e. subclinical field changes).

The stepwise protocol used for assessing the surgical field is presented in Figure 1.

![Figure 1](image)

**Figure 1.** Clinical Cancer Research Paper: stepwise protocol for surgical field assessment. Image E shows the clinically apparent tumor outlined in blue; the FVL area outlined in green; the boundary of surgical specimen in red (based on both white light and FVL); and the locations of punch biopsy sites from clinically visible tumor (red circle), from tissue showing FVL, placed directly abutting FVL boundary (green circle), and, from tissue showing FVR, placed directly abutting the boundary of surgical specimen (blue circle).

Of the 122 oral mucosa biopsies obtained from 20 surgical specimens:

- All tumors showed fluorescence visualization loss (FVL)
- For 19 of the 20 tumors, the FVL extended in at least one direction beyond
the clinically visible tumor, with the extension varying from 4 to 25 mm.

- Thirty-two of 36 FVL biopsies showed histologic change (7 squamous cell carcinoma/carcinomas in situ, 10 severe dysplasias, and 15 mild/moderate dysplasias) compared with 1 of the 66 florescence visualization retention (FVR) biopsies.

- Molecular analysis on margins with low-grade or no dysplasia showed a significant association of loss of heterozygosity (LOH) in FVL biopsies.

These findings demonstrate that the VELscope system can identify subclinical high-risk fields with cancerous and precancerous changes.

See Appendix C for a copy of this manuscript.


VELscope was used in a study of 133 patients at a community dental clinic in a “poor, medically underserved” area. The results were as follows:

- On clinical examination, 26 had clinical leukoplakia.

- Upon examination with VELscope, all 26 showed clinically significant alteration in fluorescence at the lesion site.

- Of the 6 lesions biopsied 1 presented oral cancer and 4 presented oral premalignant dysplasia.

See Appendix D for a copy of this manuscript.


The article by Poh et al appearing in the January 2007 issue of Head & Neck presents case studies involving three patients examined both under white light and with the aid of the VELscope system. The results show that the device facilitated the detection of abnormal tissue that was not apparent under white light visualization: primary dysplasia in case 1; a second primary cancer in case 2; and cancer recurrence in case 3. Representative images are presented below.
Figure 2. Representative images from the Head & Neck Paper study

See Appendix E for a copy of this manuscript.


The interim results from a longitudinal study conducted by the British Columbia Cancer Agency were presented by Poh et al during the 2007 AACR annual meeting.

- 790 lesional fields yielded a total of 2731 examinations.
- FVL was significantly correlated with the severity of histology, present in 24% non-dysplastic, 73% low-grade, 94% high-grade lesions and 96% cancers ($P < 0.0001$).
- In 14% of exams, the FVL was present at former lesion sites that were no longer clinically apparent.
- Out of 40 FVL-persistent-former lesion sites, 21 cases have developed the clinically visible lesions during follow-up. Among those biopsied (N=12), the result showed 6 high-grade, 3 low-grade and 3 non-dysplastic.
- The remaining 19 biopsies (from lesions that were still non-apparent clinically after follow-up) revealed 6 cancers, 6 high-grade dysplasia, 5 low-grade dysplasia and 2 with no dysplasia.

As concluded in the abstract, “these data [further] supports the use of the VELscope [system] as an adjunct tool to identify high-risk oral lesions.”

See Appendix F for a copy of the Abstract.

In this paper Williams et al describe the systematic approach adopted by clinics affiliated with the British Columbia Oral Cancer Prevention Program (BC OCPP) to the screening for malignant or potentially malignant oral mucosal lesions.

This approach, which the authors recommend for consideration by regular dental offices for use in daily practice, includes the routine use of fluorescence visualization (FV) as an essential component of oral cancer screening examinations.

To exemplify the justification for integrating FV into the approach adopted, the authors present a clinical case in which FV led to the discovery of dysplastic tissue lying beyond the clinically visible boundaries of a lesion (see Figure 3).

The authors go further to ascertain that, “In contrast to Toluidine blue (which stains nucleophilic tissue components, primarily DNA), [FV] detects a complex interplay of alteration to tissue structure and biochemistry that has been associated with premalignant disease and cancer at several sites.”

![Figure 3](image)

**Figure 3.** JCDA Paper. Visualization of a diffuse, nodular erythroleukoplakia at the right lateral ventral tongue in a 52-year-old former smoker. (a) The arrow indicates a clinically undifferentiated area posterior to the nodule. (b) Direct fluorescence visualization (dark brown area within the normal green autofluorescent background) shows a wider region of change. (c) Toluidine blue staining identifies an ill-defined area in addition to the posterior nodular area. (d) Histological preparation of biopsy sample from the area marked with the arrow reveals moderate to severe dysplasia. (e) Microsatellite analysis shows high-risk molecular pattern of alteration within the biopsy area.

See Appendix G for a copy of this manuscript.

This presentation summarized further results from the study reported in the Clinical Cancer Research paper (see section 5.3).

- Loss of fluorescence visualization (FVL) was noted in all 35 lesions examined (22 high-grade dysplasias/carcinoma in situ [HGLs] and 13 squamous-cell carcinoma [SCCs].)
- Nearly all HGLs (21/22; 95%) presented FVL extending 1-25mm beyond the clinical boundaries.
- Strikingly, 35% (13/37) of biopsies from FVL regions beyond the clinical boundaries of HGLs presented high-grade change upon histopathology examination. Five of them showed high-grade change beyond the conventional 10-mm tumor excision margin.

These results represent additional corroboration that fluorescence visualization has the ability to identify clinically non-apparent oral lesions.

See Appendix H for a copy of the Abstract.

5.9 “Sensitivity of direct tissue fluorescence visualization in screening for oral premalignant lesions in general practice”, Huff et al, General Dentistry, Jan/Feb 2009.

Huff et al report a retrospective comparative study in which two oral cancer screening examination protocols were performed in the same patient population. First, all patients age 12 or older visiting a private general dentistry practice for one year were given the standard oral cancer screening examinations. Then, during the following year, the same population was examined as per the same protocol, but with the addition of direct tissue fluorescence visualization (FV) using the VELscope system.

- Screening with the standard protocol alone resulted in a prevalence of mucosal abnormalities of 0.83%, none of which was found to be premalignant.
- The modified protocol (i.e., with FV) resulted in a prevalence of mucosal abnormalities of 1.3%, of which 83% were confirmed through biopsy and histopathology to be potentially premalignant epithelial dysplasia.

The authors conclude that the addition of FV to the standard oral cancer screening protocol increased the sensitivity in detection of oral potentially premalignant lesions, thus supporting the usefulness of the VELscope system in
a private practice setting for detecting such lesions in a stable low-risk population.

See Appendix I for a copy of this manuscript.


This letter is in response to another paper published in the same issue and reports on a retrospective analysis of recurrence rates for lesions excised with VELscope-guided surgery compared with conventional white-light-only assessment.

- Between 2004 and 2008, 60 patients underwent surgery to remove cancerous lesions at the British Columbia Cancer Agency in Vancouver, BC, Canada.

- The lesions were all ≤ 4cm in diameter and were treated with only surgical excision. 32 of the 60 patients had the VELscope device used to assist the surgeon in determining the appropriate margin for surgical excision and 28 had conventional white light examination alone with a 10mm clean margin surrounding the visible extent of the lesion.

- After a minimum of 12 months follow-up, 7 of the 28 (25%) patients who underwent non-fluorescence guided surgery experienced severe dysplasia or worse at the treated site. None (0%) of the VELscope-guided surgery patients had severe dysplasia at the treated site.

This report is significant in that it looks at recurrence rate. Although the initial margin study published in Clinical Cancer Research in 2006 presented impressive results showing dysplasia or even cancer in tissue around cancerous lesions that showed loss of fluorescence but were judged to be normal under conventional examination (see section 5.3 above), it didn’t address the question of whether or not the long-term outcome for the patient was affected. This 2009 report starts to demonstrate (albeit in a retrospective fashion) that long-term health of patients can be positively affected by using the VELscope to help the clinician during surgical excision.

See Appendix J for a copy of this manuscript.
5.11 “Assessment of the VELscope as an Adjunctive Examination Tool”, Huber, Texas Dental Journal, June 2009.

The investigator examined 130 patients (with a previous history of smoking) in a university clinical setting, first with a conventional examination and then with the VELscope.

- 10 suspicious lesions were identified with the conventional exam.
- No additional “occult” lesions were discovered using the VELscope.
- The VELscope was not judged to have enhanced the visualization of the lesion or played any role in altering their clinical management.
- Commonly occurring benign lesions also displayed a loss of fluorescence.

The author concludes:

- “The findings of this study raise questions concerning the utilization of the VELscope as a screening adjunct.”
- “More study is indicated to determine the true value of routine use of the VELscope in routine dental examinations.”

This study does not directly support that the VELscope performs in accordance with its Indications for Use of enhancing the visualization of oral mucosal abnormalities. It is certainly conceivable that for some patient population samples, it is possible to run into situations where the VELscope is less useful for the particular lesions that occurred. Also, the fact that the VELscope was not judged to have enhanced the visualization of any lesions or discovered any ones (maybe no such subtle lesions to be found in this particular dataset) could be a function of the expertise of the examiner with the traditional examination and also his experience and training with the VELscope device itself. The author concludes that more study is indicated and there have been numerous studies published since this one in 2009.

See Appendix K for a copy of this manuscript.


The stated purpose of the study is given in the quotation from the manuscript below:

“The purpose of our study was to evaluate the use of these two systems as adjunct aids in diagnosing lesions deemed clinically innocuous according to
conventional light examination. We also assessed the sensitivity and specificity of ViziLite and VELscope in the identification of oral dysplasia and carcinoma by independently comparing pathological examination results with those obtained with these visual screening aids.”

In a rural dental setting in India, patients were prescreened with an overhead dental light. Patients with either no lesion or with Class II lesions (those that cause suspicion of intraepithelial neoplasia or show frank evidence of malignancy necessitating immediate biopsy) were excluded from subsequent examination by the VELscope. Only those patients with Class I lesions (lesions that in the investigators' opinion required no further attention other than clinical follow-up) were examined with the VELscope. In total, 156 patients who were examined with the VELscope underwent biopsy; 11 had dysplasia and 1 had cancer. The investigators used the following definitions for a “positive” and “negative” outcome of the VELscope evaluation:

“Normal mucosa – a negative VELscope finding—appears as a bright green glow, while abnormal mucosa – a positive VELscope finding—is identified by a loss of fluorescence and appears dark.”

According to this definition, the VELscope was ‘positive’ for five dysplasias (two mild and three moderate) and the one cancer. It was ‘negative’ for the other 6 dysplasias (the breakdown in dysplasia severity is not given in the manuscript). In addition, VELscope exams were negative in 56 patients with benign lesions and positive in 88 patients with benign lesions.

The authors remark that:

“The poor sensitivity and poor positive predictive values of these devices (ViziLite, 0 percent; VELscope, 50 percent) have significant implications for dentists and physicians who attempt to rely on these aids to determine whether a lesion is benign or precancerous or cancerous.”

They conclude that:

“Although ViziLite and VELscope have been promoted as valuable adjuncts in the early detection of oral precancerous and cancerous lesions, the results of our study indicate that they do not add any benefits to a conventional screening examination involving the use of a standard overhead light.”

Given this negative portrayal of the value of the VELscope to clinicians, it must be asked: Does this study provide evidence to support that the VELscope system does not perform as per its Indications for Use?

The following observations need to be considered to help answer this question:

- The VELscope was not evaluated according to its indications for use because it was compared with surgical biopsy in its ability to identify
precancerous and cancerous lesions and differentiate them from benign lesions. In other words this is treating the VELscope like a standalone diagnostic test which is contraindicated by its labeling. This whole approach pervades the entire study: as further evidence, note the explicit identification of a “positive” and “negative” result to the VELscope exam. No such explicit identification is to be found in any of the VELscope labeling.

• Notwithstanding the above consideration, the erroneous calculation of ‘sensitivity’ and ‘specificity’ was calculated on the subset of patients with benign appearing lesions. Thus, the dire, negative consequences of the “50% sensitivity” are somewhat misleading. These patients were examined by an expert clinician in the context of a clinical study and 12 lesions were falsely identified based on a traditional exam as being Class 1 – benign. One of these was actually cancer. That the VELscope was able to show a visual anomaly as evidenced by a pronounced loss of fluorescence in at least 6 (including the one case of outright cancer) of these 12 lesions (that were otherwise considered benign) can as easily be construed as providing evidence that the VELscope is performing as per its indications for use.

• As it relates to ‘specificity’: “VELscope exams were ‘negative’ in 56 patients with benign lesions and positive in 88 patients with benign lesions.” VELscope is indicated as a visual enhancement for all types of mucosal abnormalities and does not claim, in and of itself, to discriminate between precancerous/cancerous lesions and lesions related to other forms of ‘benign’ disease. This type of discrimination relies on clinical judgment and integration of all the pieces of information at the clinician’s disposal (patient history, risk factors, medications, traditional visual and tactile head and neck examination and VELscope examination). This highlights the fallacy of trying to isolate any one element of the diagnostic process as a standalone predictor.

• Finally, it must be pointed out that, since there is no well-defined ‘positive’ or ‘negative’ definition for an outcome of a VELscope examination, one must question the clinical interpretations of positive and negative as reported on in this study. Indeed, since the authors failed to demonstrate an understanding of the appropriate intended use of the device, it definitely calls into question these self-defined ‘positive’ and ‘negative’ outcomes.

In conclusion, the evidence in this study does not provide substantial evidence that the VELscope system does not perform as per its Indications for Use. Indeed, there is evidence that for 6 of 12 precancerous/cancerous lesions judged to be benign clinically, the VELscope provided valuable additional information that enhanced the visualization of these abnormalities.

See Appendix L for a copy of this manuscript.

126 patients with white and red lesions suggestive of oral potentially malignant disorders (OPMD) were enrolled in a study at Oral Medicine Clinics at King’s and Guy’s Hospitals, London. After a complete visual and autofluorescence examination (with VELscope) all the lesions were biopsied. Clinically, the lesion breakdown was as follows:

- 70 patients had oral leukoplakia/erythroplakia
- 23 patients had lichen planus
- 9 chronic hyperplastic candidiasis
- 13 had frictional keratosis
- 2 had oral submucous fibrosis

Loss of fluorescence was observed in 105 of the 126 lesions (126). Following biopsy, 44 had oral epithelial dysplasia (29 mild, 8 moderate and 7 severe). The ‘sensitivity’ and ‘specificity’ of the VELscope was reported as 84.1% and 15.3% respectively.

The authors conclude: “While VELscope was useful in confirming the presence of oral leukoplakia and erythroplakia and other oral mucosal disorders, the device was unable to discriminate high-risk from low-risk lesions.”

In evaluating the relevance of this study on the performance of VELscope device according to its Indications for Use, one should consider the following:

- The VELscope was not evaluated as per its Indications for Use – it was evaluated as a standalone diagnostic test, which it is NOT (given that the results of the VELscope examination must be considered within the context of the other clinical information) and with well-defined positive and negative outcomes. The positive outcome was defined as “loss of fluorescence” but this is an inappropriate standalone predictor – some types of normal tissue (e.g. tonsillar or lymphoid tissue, attached gingiva, fungiform papillae on the dorsal surface of the tongue, anterior tonsillar pillars). Certain patterns of loss of fluorescence is suggested by the VELscope labeling as increasing suspicion of an abnormality but only when observed in certain clinical contexts. This labeling though is equally clear of all the other types of causes unrelated to cancer or dysplasia that can give rise to a loss of fluorescence.
- Once again, the VELscope is being evaluated as a test designed to be specific to dysplasia and oral cancer whereas the Indications for Use claim it is a visual enhancement for the detection of all types of abnormal tissue including dysplasia and cancer. Concluding that the device (by itself) is “unable to discriminate high-risk from low-risk lesions” while of potential academic interest, is not relevant as per its intended use. It also comes as
no surprise to the manufacturer and moreover is anticipated by and discussed in the VELscope labeling (e.g. VELscope Vx Step-by-Step Examination Guide). In addition, it does not address how the fluorescence examination taken together with the traditional protocol (as opposed to an alternative to it) can enhance the ability of the clinician to discriminate between high-risk and low-risk lesions.

- The pool of patients studied is one where suspicious lesions have already been identified by experts and so does not address one of the primary modes in which the VELscope is designed to be used – by the general practitioner on the general population or higher risk sub-population to enhance the visualization of, and thus help discover, lesions that might otherwise have been overlooked.

- The fact that the large majority of suspicious (as pre-judged by experts in oral medicine) lesions showed a loss of fluorescence is encouraging and does not call into question the Indications for Use of the VELscope as a visual enhancement to a traditional examination.

See Appendix M for a copy of this manuscript.


62 high-risk patients were examined with the VELscope device and a ‘sensitivity’ of 100% and ‘specificity’ of 80.8% was reported as judged by the presence or lack thereof of loss of fluorescence. This paper has some worthwhile discussion of the importance of understanding the types of benign or non-cancerous lesions that can also present as a loss of fluorescence. Also, there are some good clinical cases and additional discussion regarding the effects of porphyrins from bacteria and the presence of extensive amounts of keratin in verrucous leukoplakia and verrucous carcinoma.

See Appendix N for a copy of this manuscript.


Adding a VELScope examination to the routine clinical examination of 620 routine dental patients at the University of Washington dental clinic resulted in detection of changes not seen with white light examination in a significant number of patients (11.1%). Of these a small but important number (1.5% of the 620 patients) were found to have otherwise undetected persistent changes representing inflammatory lesions or potentially dangerous oral dysplasia. The complete breakdown of the lesions not detected by the traditional white light exam is as follows:

- 3 lesions: mild dysplasia
• 2 lesions: mild to moderate dysplasia
• 2 lesions: lichen planus
• 2 lesions: inflammatory of unknown origin

This is a very significant study pertaining to the use of the VELscope by general practitioners as it directly assesses the value of adding the VELscope examination to the conventional head and neck exam for routine (as opposed to high-risk) patients. In addition, the clinicians involved in the initial examination were trained dental students not specialists or otherwise experts in oral medicine. Therefore this study highlights the potential utility of the device for improving the ability of general practitioners in their dental practices to detect unhealthy mucosal conditions (not just premalignant or cancerous tissue) that might otherwise have remained undiscovered using only the traditional head and neck exam.

See Appendix O for a copy of this manuscript.


This is a study that enrolled 112 patients referred by general practitioners to an oral medicine specialist for further assessment of white or mixed red/white lesions that were clinically suspicious. This is somewhat of a unique study because although it evaluated the use of the VELscope as a standalone predictor (contrary to the Indications for Use) it also tried to evaluate and quantify performance of the combined use of a conventional oral examination together with a VELscope examination. In addition, a subjective qualitative analysis was attempted by asking questions of the investigator for the particular lesions such as:

• Did VELscope examination enhance visualization?
• Did VELscope examination uncover a new lesion?

To summarize the results:

• Of 118 biopsied lesions, 80 displayed loss of fluorescence and 38 retained fluorescence.
• In the group of lesions that lost fluorescence, there were 19 dysplastic or cancerous lesions:
  o 13 mild dysplasia
  o 2 moderate dysplasia
  o 1 severe dysplasia
  o 3 OSCC
• In the group that retained fluorescence there were 8 cases of dysplasia:
  o 7 mild dysplasia
• 1 moderate dysplasia
  • The VELscope enhanced the visualization of the lesions in 34.7% of cases.
  • When treated as a standalone diagnostic test for dysplasia/cancer with ‘positive’ defined as a loss of fluorescence that could not be blanched upon application of diascopic pressure, and ‘negative’ as retained fluorescence and regained fluorescence with diascopic pressure, the sensitivity and specificity of the VELscope is 30% and 63% respectively.
  • Conventional oral examination (COE) had a corresponding sensitivity and specificity of 25% and 82% respectively.
  • Combined use of COE and VELscope (significantly more consistent with an adjunctive visualization tool as per the Indications for Use) had a corresponding sensitivity and specificity of 46% and 68% respectively.
  • Use of the VELscope led to the discovery of 5 lesions that were undetected by COE, one of which turned out to be moderate dysplasia.

The authors conclude:

“VELscope seems to be of use at aiding the visualization of potentially malignant, malignant, and inflammatory conditions; however, it cannot accurately or consistently differentiate between them, even in the hands of experienced oral medicine specialists. VELscope is a useful clinical tool for visualizing abnormalities of the oral mucosa, but cannot provide a definitive diagnosis as to the presence or otherwise of dysplastic tissue change. Its use requires a significant understanding of mucosal pathology, and interpretation of results requires skill and training. Although clinical inspection and histopathology have certain limitations, they remain the gold standard for determining a definitive diagnosis.”

Like most of the academic research on the VELscope, this study primarily tries to assess the VELscope’s usefulness as a tool to aid in the assessment of dysplasia and cancer. It highlights the fact that VELscope should not be used as a diagnostic test by itself and does not reliably differentiate between dysplastic/cancerous lesions and inflammatory conditions. It does however demonstrate that combining a conventional oral examination with the use of the VELscope does lead to an increased sensitivity to dysplasia. It also supports the fact that the VELscope enhances the visualization of oral lesions. It also stresses the importance of clinical judgment and the fact that histopathology remains the gold standard for diagnosis. All of the above considerations and limitations are consistent with and do not run contrary to the VELscope’s Indications for Use and labeling.

In general, it supports that the VELscope performs as per its Indications for Use and highlights the important limitations of its use consistent with those indications and the accompanying documentation with the system in general.

See Appendix P for a copy of this manuscript.

78 patients were included in the study with lesions all having been either clinically diagnosed with SCC or being suspicious lesions requiring histological evaluation for definitive diagnosis. An analysis of the performance of the VELscope was made by treating either a complete loss of fluorescence or the presence of red mucosal fluorescence as an indicator for dysplasia or SCC and then comparing the results with the clinical diagnosis of an expert specialized examiner, using histological evaluation of surgical biopsy as the gold standard.

The key results were as follows:

- For the detection of SCC or dysplasia, using a “low autofluorescence signal” as the definition of a ‘positive’ test, the sensitivity and specificity were 94% and 16% respectively.
- The corresponding sensitivity and specificity of a red autofluorescence signal as a ‘positive’ indicator was 22% and 16% respectively.

The authors conclude:

"With a high sensitivity and NPV, but a low specificity and PPV, oral mucosal lesions could be detected by autofluorescence The autofluorescence examination, however, is no able to differentiate between benign and malignant oral lesions. Red autofluorescence should be an indication for scalpel biopsy due to a high PPV for cancer."

Although interesting for its evaluation of red fluorescence (usually thought to be associated with the presence of bacteria which can sometimes get entrapped on the non-smooth textured surface of abnormal lesions) this study does not attempt to evaluate the VELscope according to its Indications for Use. In a population with a high prevalence of cancerous lesions (30 out of the 78 lesions were SCC) already pre-identified by a conventional oral examination, it attempts to evaluate the device as a standalone diagnostic predictor. The high sensitivity for loss of fluorescence is affected by the high prevalence of SCC, and the low specificity is typical of the well-understood loss of fluorescence associated with inflammatory lesions.

The Indications for Use and labeling of the VELscope system do not support use of the device as a standalone diagnostic test for dysplasia/SCC and specifically remind the user that histological evaluation of biopsies is the gold standard for diagnosis. The results of this study have limited applicability to use of the VELscope by a general practitioner as an adjunctive aid to a traditional exam.

See Appendix Q for a copy of this manuscript.

123 patients were examined with conventional white light as well as autofluorescence examination using the VELscope. Biopsies were taken from lesions considered to be clinically suspicious.

- Out of the 123 patients, there were 6 biopsy-confirmed cases of dysplasia.
- With dysplasia defined as a positive outcome, and a persistent loss of fluorescence in a lesion (including a 2 week follow-up visit filter to help identify lesions that were of an acute inflammatory origin) defined as a positive test result, the investigators computed a sensitivity and specificity of the combined use of the VELscope system and a white light examination as 100% and 74% respectively.
- The sensitivity and specificity of the white light examination alone was computed to be 17% and 97% respectively.
- This means that 5 of the 6 dysplastic lesions were discovered using the VELscope examination but missed by the prior white light examination.
- The reduction in specificity was a result of the usual phenomenon of other types of oral disease (such as lichen planus) showing a loss of fluorescence because of the inflammatory response.

The authors conclude:

“To conclude, VELscope is a simple, noninvasive examination test of the oral mucosa with the ability to help locate malignant oral lesions and find the right location for a biopsy. However, its results have to be interpreted carefully, and a good examination protocol and documentation is very important to decrease false-positive results. It cannot replace histological evaluation of the oral tissue as a gold standard.”

Notwithstanding that the investigators evaluate the device as if it were a diagnostic test specific to dysplasia and oral cancer (contraindicated by the VELscope labeling), it was encouraging to see the evaluation consider the combined results of both the traditional white light examination and the VELscope examination (which is in keeping with the definition of an adjunctive device). The authors’ conclusion is a good one and fully consistent with the VELscope’s indications for use and labeling. Most notably, without the use of the VELscope, 5 of the 6 dysplasias present in the population would have been missed. This clearly supports the claim that the VELscope enhances the visualization of oral mucosal abnormalities (of which dysplasia is an important subset).

See Appendix R for a copy of this manuscript.

5.19 “Fluorescence Visualization Devices in General Dentistry: Seeing the Big

Although not an independent clinical study, it is maybe useful to also include in this report an article written by David Morgan, Chief Science Officer of LED Dental Inc. (the author of this clinical evaluation report as well) since it discusses some clinical examples not found anywhere else in the published literature and explicitly illustrates many of the concepts discussed in this report.

See Appendix S for a copy of this manuscript.

6 Future Clinical Research

6.1 NIDCR supported & BCCA-Led Multi-Centre Lesion Margin Study

This is a prospective, multi-centre, randomized controlled clinical trial with the main objective being to demonstrate that fluorescence-guided surgery for the excision of premalignant and malignant lesions (severe dysplasia, carcinoma-in-situ and squamous cell carcinoma (T1 or T2)) yields improved recurrence-free survival compared to the use of conventional white light assessment alone. The trial is anticipated to take 5 years in total, 2 years for the accrual of 200 patients and 3 more years of follow-up. The study will have 2 arms: 100 patients in the fluorescence-guided surgery arm and 100 patients in the control arm (conventional white light assessment of surgical margin). There are at least 8 different trial sites across Canada. VELscope Vx units have been provided to the organizers of the study to perform the fluorescence assessments of the surgical fields containing the lesions. As of May, 2011 the sites are starting to enroll patients.

See Appendix T for an overview of the protocol of this clinical trial obtained from the NIH site ‘clinicalTrials.gov’ which is a registry and results database of federally and privately supported clinical trials conducted in the United States and around the world.

7 Adverse Events, Safety Concerns & Post-Market Surveillance

In reviewing the manuscripts included in this report there were no adverse events or safety concerns reported concerning the use of the VELscope/VELscope Vx systems. In many cases, LED Dental Inc. had direct communications with the investigators before, during and after the study or clinical use of the device and there were no safety concerns or adverse events reported this way either. In any event, these would also have been documented in the company’s customer complaint or CORE system. This absence of adverse events is consistent with the low-risk profile of the device as determined and documented in the VELscope and VELscope Vx Risk Management Files. An annual review of reported safety-related adverse events associated with the VELscope and VELscope Vx systems is
conducted and documented in the associated Risk Management Files for the devices. This process not only includes review of adverse events reported in the literature, but also though the company’s own CORE system as well as any safety issues discovered via active postmark surveillance such as customer surveys. Again, the lack of safety-related adverse events as reported in the published literature reviewed is consistent with these annual reviews.

Over the years the most notable adverse events associated with the use of either the VELscope or VELscope Vx systems has been from:

1. Inadvertent patient eye exposure to the bright blue excitation light from the original VELscope system. Since according to the device indications, the patient must wear safety glasses to block the light, this would not have occurred had the patient been wearing the safety glasses. There have been isolated anecdotal accounts of users inadvertently accidentally shining the light from the VELscope into their own eyes. This causes some transient discomfort but there have been no corresponding reports of permanent eye damage. It should be stressed that, as documented in the corresponding Risk Management Files, all models of the VELscope meet international safety standards for eye exposure.

2. Rare and unusual reactions of intra-oral tissues after the administration of a VELscope exam. There have been 4 or 5 cases of the phenomena of ‘dry mouth’, patient perception of a slight burning sensation, possibly some minor tissue swelling (as documented in the company’s CORE database). There were no corresponding reports of serious injury. These reports were all associated with the use of the original VELscope system. Although rare, and difficult to ascertain for certain that the tissue reaction was definitely associated with tissue exposure to blue light, stronger wording was included in the VELscope Vx Operation Manual to warn of the possibility of photosensitive reactions. Finally, it should be once again noted that as documented in the Risk Management Files, all models of the VELscope meet international safety standards for tissue exposure to blue light.

As stated above, annual reviews of product safety issues are conducted through the company’s post market surveillance activities. These reviews are documented in the Risk Management Files:

- RMF 0102 VELscope V2 Risk Management File
- RMF 03 VELscope Vx Risk Management File

Any new product safety concerns or issues discovered as part of this process will also be included as an update to this report.
8 Conclusion

The VELscope Vx and earlier generation VELscopes are qualitative tools intended to enhance the visualization of oral mucosal abnormalities, and they have identical Indications for Use. The VELscope Vx and previous versions also have similar excitation and emission spectra and power, and they are expected to have equivalent performance. This rationale in combination with clinical comparisons between the visualization of different types of lesions using VELscope Vx versus the older VELscope are an appropriate way to assess that the clinical utility of the newer generation VELscope Vx is comparable with that of the original. From close inspection of the clinical photographs of the lesions in this report, it can be concluded that there is no significant difference between the performance of the VELscope Vx with the original VELscope. Thus, the published clinical studies on the earlier generation VELscope (that have been documented in the VELscope design validation reports) can be considered also to apply to the clinical performance of the VELscope Vx.

The twenty clinical reports cited and summarized in this document and included in the Appendices validate the use of the VELscope Vx as a qualitative tool in helping detect oral mucosal abnormalities that can be subsequently diagnosed using biopsy. The clinical reports further validate the use of the VELscope Vx in helping identify suitable surgical margins during removal of cancerous lesions. Therefore, it can be concluded that there is adequate clinical evidence to support that the VELscope Vx performs in accordance with its Indications for Use.
## Document History Page

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Appendix A
Simple device for the direct visualization of oral-cavity tissue fluorescence

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Abstract. Early identification of high-risk disease could greatly reduce both mortality and morbidity due to oral cancer. We describe a simple handheld device that facilitates the direct visualization of oral-cavity fluorescence for the detection of high-risk precancerous and early cancerous lesions. Blue excitation light (400 to 460 nm) is employed to excite green-red fluorescence from fluorophores in the oral tissues. Tissue fluorescence is viewed directly along an optical axis collinear with the axis of excitation to reduce inter- and intraoperator variability. This robust, field-of-view device enables the direct visualization of fluorescence in the context of surrounding normal tissue. Results from a pilot study of 44 patients are presented. Using histology as the gold standard, the device achieves a sensitivity of 98% and specificity of 100% when discriminating normal mucosa from severe dysplasia/carcinoma in situ (CIS) or invasive carcinoma. We envisage this device as a suitable adjunct for oral cancer screening, biopsy guidance, and margin delineation.

Keywords: autofluorescence; fluorescence imaging; oral cancer; squamous cell carcinoma.

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1 Introduction

Oral squamous cell carcinoma (SCC) is believed to progress from oral premalignant lesions (OPLs)—hyperplasia, increasing degree of dysplasia (mild, moderate, and severe) into carcinoma in situ (CIS), and finally to invasive SCC (from stages I to IV). This disease is a source of substantial morbidity and mortality with over 275,000 new cases and 127,000 deaths worldwide1 in 2002. In the United States, it is estimated that there will be2 29,370 new cases of oral cancer and 7320 deaths in 2005. Cancer stage at the time of the diagnosis is directly related to the disease mortality: early detection enables minimally invasive treatment procedures. It also is related to the disease mortality: 5-yr survival rate is 81% for early stages of oral SCC versus 30% for late-stage disease.2 However the 5-yr survival rate has remained dismal with little improvement over the last 30 yr (<50%), mainly due to the fact that a significant proportion of these cancers are still diagnosed late2 (stages III and IV).

Ironically OPLs and early SCC have known clinical presentations, mostly as leukoplakia (white patches) and sometimes as erythroplakia (red patches). These lesions are present in a site that is readily accessible for visual inspection, possibly as part of an annual dental visit. Additionally there are known high-risk populations (e.g., elderly heavy smokers) that should be examined regularly. The main limitation is that
differential diagnosis of OPLs from the much higher incidence of lesions resulting from nonspecific inflammation and irritation (that also appear as white or red patches) is challenging even for experienced specialists. Patients may be reluctant to submit to an invasive and sometimes painful biopsy when their dentists’ confidences as to the nature of the lesions is low. Furthermore, there is increasing awareness that many OPLs and early SCC are clinically occult and not visible under white-light examination. Together, these difficulties can result in a failure to biopsy and hence a delay in the diagnosis, since the actual assignment of risk requires a biopsy to determine the presence and degree of dysplasia/invasion. The development of adjunct tools to facilitate the noninvasive screening of high-risk oral lesions in real time has the potential to significantly improve our ability to reduce the dismal morbidity and mortality of oral cancer.

While white-light visualization can perceive only a fraction of the spectral characteristics that differentiate diseased tissue from its normal counterpart, optical methods, particularly those based on fluorescence imaging and spectroscopy, will likely improve our ability to detect tissue changes, such as OPLs and early cancer. Recent studies have shown great promise in the screening and diagnosis of precancers in the lung, uterine cervix, skin, and oral cavity.

In this paper, we present a simple field-of-view device for the direct visualization of tissue fluorescence in the oral cavity. Instrumentation for fluorescence imaging requires a light source, excitation and emission filters, and a means of detection. The fluorescence can be detected and visualized directly by a human observer, as intended by the device described in this paper, or recorded by a camera and visualized indirectly. Recently, fluorescence imaging studies for the early detection of oral malignancies have employed indirect visualization—either photographic film or a sensitive or intensified CCD camera. De Veld et al. provide a good review of in vivo autofluorescence spectroscopy and imaging for oral oncology. The methods of excitation, emission, and detection employed in several recent studies are briefly summarized here.

Onizawa et al. used a custom UV-flash photography system to record porphyrin-like fluorescence in the oral cavity. Fluorescence was excited by the 360-nm spectral peak of the flash lamp and fluorescence was recorded on photographic film using a 480-nm long-pass filter. Lesions were classified based on the intensity and color of the fluorescence recorded on the film. The authors reported 91% sensitivity and 84% specificity for discriminating benign from malignant lesions (and 94% sensitivity and 96% specificity for discriminating SCC and dysplasia from benign lesions).

Other groups have used commercially available autofluorescence systems and sensitive or intensified CCD cameras to image oral-cavity fluorescence. Betz et al. and Paczona et al. used a Storz endoscopy system configured for white-light and autofluorescence imaging. In autofluorescence mode, illumination from a xenon arc lamp at 375 to 440 nm excited fluorescence, which was detected by a sensitive color CCD camera through a 515-nm long-pass filter. Video frame integration for as long as 2 s was required. The authors report a similar loss of fluorescence intensity at neoplastic lesions. Kulapeditharam and Boonkitticharoen used the Xillix laser-induced autofluorescence endoscopy (LIFE) system in their study of head and neck cancers. The LIFE system, designed for fluorescence bronchoscopy, employs excitation at 442 nm and dual image-intensified cameras for detection in the green (480 to 520 nm) and red (>630 nm) emission channels. Lesions were classified based on the green-to-red fluorescence ratio displayed as a pseudocolor image. The authors found LIFE to be an effective and reliable tool for detecting head and neck cancers and reported increased sensitivity and specificity compared to white-light imaging.

More recently, Svishtun et al. proposed a system for the direct visualization of oral cavity fluorescence. In their device, excitation light is provided by a handheld illuminator and tissue fluorescence is observed along an axis slightly inclined from the illumination axis using special glasses. In their study, tissue fluorescence of freshly resected oral tissue was observed visually and photographed at specific excitation wavelengths suggested by previous studies of fluorescence spectroscopy and optimal visual perception. Perceived tumor margins, as determined from the fluorescence images and not observed directly through the viewing glasses, were correlated with histopathology. The sensitivity and specificity were 91 and 86% for the discrimination of normal tissue from neoplasia at the best excitation wavelength. The sensitivity and specificity were 75 and 43% for the same task using white-light images.

In contrast, the device presented in this paper employs coaxial optical pathways for excitation and emission. By employing coaxial illumination and imaging we hope to reduce inter- and intraoperator variability. In addition, we also present results based on the direct visual impression of oral cavity fluorescence rather than the indirect visualization from an image recorded on photographic film or detected by a CCD camera.

In an earlier version of the direct visualization device presented in this paper, tissue fluorescence was shown to more accurately estimate the histological margins of basal cell carcinoma (BCC). In a study of patients with BCC, tumor margins were delineated under white light and then again using direct fluorescence visualization. Fluorescence visualization more accurately estimated the histological margins of the BCC.

There is clearly a need for a simple cost-effective screening device for the early detection of oral premalignant lesions. In this paper, we describe such a device, which aids in the direct visualization of tissue fluorescence for the early identification of precancers in the oral cavity. The study objective was to test this real-time device for its ability to discriminate high-risk OPLs and invasive SCCs from normal oral mucosa.

2 Material and Methods

2.1 Device

The device for direct fluorescence visualization (direct FV), illustrated schematically in Fig. 1, consists of a bench-top light source coupled to a handheld unit for visualization. The light source (X-Cite 120, EXFO) used a 120-W metal-halide arc lamp with integral elliptical reflector optimized for near-UV/blue reflection. The power coupled into the light guide was adjustable in steps by rotating a variable-pinhole wheel. Light was coupled from the light source to the handheld unit by a 0.59—numerical aperture (NA), 3-mm-diam liquid light guide (Lumatec, Germany). The handheld unit projected ex-
The emission filter was a 475-nm long-pass bandpass filter centered at 425 nm. A two-element lens system divided the fluorescent light spectrum into red and green components. Photographs of the light source and handheld unit produced a spot 44 mm in diameter on the tissue at a distance of 100 mm. At maximum power the peak irradiance was nearly collimated. A dichroic mirror passed the green-red fluorescent light and rejected the blue excitation light while the notch filter divided the fluorescent light spectrum into red and green components. Photographs of the light source and handheld unit are shown in Fig. 2.

The excitation and emission wavelengths were selected based on spectroscopic data and empirical measurements from previous unpublished studies. The excitation filter was a 60-nm bandpass filter centered at 425 nm (Chroma D425/60×) and provided an excitation spectrum composed primarily of the 405- and 436-nm peaks of the metal-halide lamp. The emission filter was a 475-nm long-pass (Schott GG475-3), while the center wavelength and bandwidth of the notch filter were chosen empirically to maximize the perceived color difference between normal and abnormal mucosa. The upper end of the emission passband was determined by the sensitivity of the human visual system.

Photographs of tissue fluorescence were acquired for documentation only and were not used for the classification of lesions. Photographs were acquired using illumination from the direct FV system and a digital SLR (single lens reflex; Fuji FinePix S2 Pro) equipped with a long-pass filter (Schott GG475-3). The SLR was equipped with a 105-mm f/2.8 macro lens (Nikkor Micro) and a ring flash (Nikon Macro Speedlight SB-29s) for white-light images.

2.2 Patients and Direct FV

Consenting patients with a history of biopsy-confirmed oral dysplasia or SCC were recruited from the Oral Health Study, an ongoing longitudinal study of oral leukoplakia at the British Columbia Cancer Agency (BCCA). The study was approved by the Institutional Review Board of the BCCA. These patients were referred primarily by community dentists to the Oral Dysplasia Clinic and the Head and Neck Oncology group at the BCCA for assessment and care.

Each follow-up visit for these patients involved an assessment, under white light, of the oral mucosa to identify new clinical lesions or alterations to previously identified clinical lesions. Particular attention was paid to sites of previous lesions (i.e., sites of prior oral SCC). After turning off the room light, the oral cavity was viewed with direct FV. The clinicians would then decide whether the oral lesions required biopsy based on standard clinical features (patient history, clinical appearance, and toluidine blue staining results\(^{25}\)) and not based on the direct FV examination. For the biopsied lesions, histopathological reviews were conducted by oral pathologists in the Provincial Oral Biopsy Service at the Vancouver Hospital and Health Sciences Center (CP and LZ) and a histological diagnosis was assigned to each lesion. Since the objective here was to verify the effectiveness of the direct FV device in differentiating high-risk OPLs and invasive SCC from normal oral mucosa, we assessed the association with direct FV changes in the oral mucosa of biopsy-confirmed sites of normal and severe dysplasia, CIS, and invasive SCC. Fifty oral biopsies from 44 patients were included in this study.

3 Results

3.1 Device

The cone of blue excitation light emitted from the handheld unit produced a spot 44 mm in diameter on the tissue at a distance of 100 mm. At maximum power the peak irradiance at the tissue was 100 mW cm\(^{-2}\).

3.2 Direct FV and Pathology

Under direct FV, the normal oral mucosa emits various shades of pale green autofluorescence [Fig. 3(b)]. Clinical lesions that retained the normal green autofluorescence under direct FV were classified as lesions with FV retention (FVR). Tissue that showed a distinct reduction in the normal pale green and appeared as dark green to black was classified as FV loss (FVL) [Figs. 3(d) and 3(f)]. This assessment involved a comparison of the lesion site with both adjacent tissue and, as an anatomical control, with tissue on the contra lateral side.
Fig. 3 Photographs of three cases. White-light images are presented on the left and fluorescence images on the right. The top row illustrates normal mucosa of the ventral tongue—this healthy tissue has a pale-green fluorescence. The middle row shows the ventral tongue of a patient with an OPL, which when biopsied was confirmed to be a severe dysplasia. Arrow in panel (d) indicates region of biopsy and FVL. Bottom row shows a clinically occult lesion under white light, panel (e), and a corresponding dark patch (indicated with arrow) with FVL under direct FV.
Table 1 Correlation of direct FV results with lesion histopathology.

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*2 X 2 chi squared of normal versus abnormal pathology (severe dysplasia, CIS, and SCC) and direct FV status.

As shown in Table 1, of the 50 biopsy samples, 7 samples were FVR, and 43 samples were FVL. None of the 6 samples with a histological diagnosis of normal showed loss of FV, whereas 91% of high-grade preinvasive lesions (severe dysplasia and CIS) and 100% of invasive SCC showed loss of FV (P < 0.0001, Fig. 3). Using histology as the gold standard, the device achieved a sensitivity of 98% and specificity of 100% when discriminating normal lesions from high-risk OPLs and invasive SCC.

4 Discussion

These biopsy-confirmed preliminary results suggest that this direct FV device has potential as a simple, cost-effective screening device for the early detection of oral premalignant lesions. As in other sites, oral tissue autofluorescence characteristics seem to include information associated with the histopathological organization of the epithelial tissue.

Significant progress has been made in understanding the mechanisms responsible for endogenous fluorescence from epithelial tissues and how this fluorescence changes with dysplastic progression. Fluorescence detected at the tissue surface is a function of tissue morphology and biochemistry. The intrinsic fluorescence, due to naturally occurring fluorophores in the epithelium and stroma, is modified by local tissue morphology through absorption and scattering, first during excitation, and then during emission. In general, the absorption and scattering modify the intensity and spectral distribution of the detected fluorescence.

The fluorophores of interest here are those that excite in the blue and have properties that have been spectroscopically correlated with dysplastic progression. The reduced form of nicotinamide adenine dinucleotide (NADH) and the oxidized from of flavin adenine dinucleotide (FAD) are important fluorophores that are good indicators of cellular metabolism. It has been shown that fluorescence intensity due to NADH increases with dysplastic progression and that of FAD decreases. Maximum NADH fluorescence occurs at 340-nm excitation and 450-nm emission (but NADH is not interrogated by the device described here), while that of FAD occurs at 450-nm excitation and 515-nm emission and is interrogated by this device. Another source of autofluorescence originates in the submucosa from collagen cross-links and has been shown to decrease in the immediate vicinity of dysplasia. This loss of fluorescence is generally attributed to changes in collagen biochemistry, possibly due to the breakdown of the extracellular matrix by dysplastic cells. One hypothesis is that matrix metalloproteinases (MMP) expression in host stromal cells and the consequent remodeling of the extracellular matrix is induced by altered signaling from dysplastic epithelial cells. The excitation and emission bands of collagen cross-links are much broader than those of NADH/FAD, probably due to the contribution of several different fluorophores to the overall spectrum. Collagen yields maximum fluorescence at 340-nm excitation (420-nm emission) and has significant fluorescence when excited between 410 and 470 nm. In this region, the emission maximum continuously shifts to the red from 475 nm at 410-nm excitation to ~540 nm at 470-nm excitation. Collagen-generated signals likely make up a significant fraction of the autofluorescent light detected by this device.

Tissue morphology affects fluorescence via absorption and scattering of the excitation and emission signals. The morphological changes that accompany dysplastic progression also affect the absorption and scattering properties of the tissue, which in turn modifies the fluorescence. It has been shown that nuclear changes observed during dysplastic progression increase nuclear scattering in cultured cells and cells from cervical biopsies measured ex vivo. The increased microvascularization that accompanies carcinogenesis leads to increased light absorption at 420 nm due to a higher hemoglobin concentration.

Based on the present knowledge of the origins of fluorescence and its change with dysplastic progression, we believe that the loss of fluorescence associated with dysplastic progression in the current device, which employs excitation from 400 to 460 nm and emission >475 nm, is primarily due to breakdown of the collagen matrix and increased hemoglobin absorption. Secondary to these effects is increased scattering in the epithelium, epithelial thickening, and a decrease in FAD concentration.

Fortuitously, studies by other researchers have identified excitation and emission wavelengths very similar to those employed by the present device for the optimal discrimination of normal and abnormal tissue. In a fluorescence spectroscopy study of 343 oral sites from 76 patients, Heintzelman et al. determined that the optimal excitation wavelengths for detecting neoplasia were 350, 380, and 400 nm. In a subsequent paper, Utzinger et al. point out that the response of the human eye should be considered when choosing the excitation and emission bands. Using the Heintzelman et al. data, the authors show that abnormal tissue is optimally perceived with excitation between 420 and 440 nm. They also suggest observing fluorescence emission in a 50-nm band centered at 515 nm to increase the perceivable contrast between normal and abnormal tissue. Svistun et al. extended the previous work by photographing freshly respected oral tissue using the
excitation wavelengths supported by the previous two studies (350, 380, 400, and 440 nm) and an emission band between 500 and 560 nm. The best sensitivity and specificity were achieved at 400- and 440-nm (our device uses 440- to 460-nm) excitation and the least favorable results were obtained at 340 nm.

Note that previously, the complex devices that record tissue fluorescence required very sensitive cameras or special tripods to reduce motion artifacts during long exposures. CCD-based instruments often incorporated postprocessing hardware to pseudocolor the final image for display. While this improves the contrast between normal and abnormal tissue it increases the cost and complexity of the instrument, making it unacceptable for large-scale deployment for a screening application. Note that all of the devices with similar performance discussed in the De Veld review employed only indirect visualization for the classification of lesions. In contrast, our simple, robust device facilitates the visualization of oral cavity fluorescence directly by a human observer. In addition to its application in precancer screening, this simple device could also be employed for biopsy guidance and margin delineation during surgical resection.

5 Summary

A simple field-of-view device for the direct visualization of tissue fluorescence was presented. The handheld unit was designed for the detection of high-risk oral premalignant lesions and SCC; however, the same instrument is easily translated to other organ sites. The design employs coaxial excitation and emission to reduce inter- and intraobserver variability.

Using histology as the gold standard for 50 sites, the device achieved a sensitivity of 98% and specificity of 100% when discriminating normal from high-risk OPLs and invasive SCC. It is envisioned that such a device would be used as an adjunct to conventional white-light screening to increase the sensitivity of white-light screen alone but not reduce the specificity.

Acknowledgments

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Detecting Oral Cancer: A New Technique and Case Reports
John C. Kois, DMD, MSD, and Edmond Truelove, DDS, MSD

Until now it has been difficult to determine which abnormal tissues in the mouth are a cause for concern. In the United States it is estimated that there were 29,370 new cases of oral cancer and 7,320 related deaths in 2005.1 The disease morbidity is directly related to the cancer stage at the time of diagnosis. Consequently, early detection impacts disease mortality and facilitates minimally invasive treatment procedures. Oral squamous cell carcinoma (SCC) is believed to progress from oral premalignant lesions (OPLs) to hyperplasia, increasing dysplasia (mild, moderate, and severe) into carcinoma in situ (CIS), and finally to invasive SCC (from stage I to IV). Unfortunately, the 5-year survival has remained very poor, with little improvement over the last 30 years (<50%), mainly because a significant proportion of these cancers are still diagnosed late (stage III and IV).2

The American Dental Association states that 60% of the US population sees a dentist every year. This obviously provides a potential to include cancer screenings and detection of oral cancer in its early stages. Unfortunately, published studies indicate that currently less than 15% of those who visit a dentist regularly report having had an oral cancer screening. It is now commonplace for women to get an annual PAP smear for cervical cancer or a mammogram to check for breast cancer. In addition, many men at risk receive an annual prostate-specific antigen (PSA) test and digital rectal exam for prostate cancer. These screening efforts have been possible due to public awareness of the value of catching cancers in their earliest forms as well as effective technologies for conducting the examinations. Oral cancer is not different in this regard. In fact, it is potentially easier to obtain patient compliance because unlike many other cancer screening procedures, there is no invasive technique necessary, no discomfort or pain involved, and it can be very cost-effective. Consequently, our dental examinations, when they are properly performed and include screening for oral cancer, will save lives.

There has been a tendency to check these lesions over an extended period of time, which may allow a SCC to grow into later-stage disease, with consequent poorer prognosis and increased morbidity. Therefore, there are 2 separate issues: detection and diagnosis. Detection is the result of thorough visual and manual examinations of all soft tissues of the mouth. This includes manual extension of the tongue to examine its base, a bimanual palpation of the floor of the mouth, and digital examination of the borders of the tongue and the lymph nodes surrounding the oral cavity and in the neck. New detection aids, including lights, dyes, and other techniques have appeared in the marketplace.

LED Medical Diagnostics (LED MD) is a Vancouver-based medical company that has developed an imaging device to assist dentists in screening for early tissue changes (OPLs) that can lead to cancer. In association with scientists at the British Columbia Cancer Agency (BCCA), LED MD jointly developed the VELscope (Figure 1). The VELscope integrates 4 key elements: illumination, sophisticated filtering, natural tissue fluorophores, and the power of human optical and neural physiology. The VELscope has the potential to overcome many of the obstacles presented by conventional methods for screening and aid in the detection of mucosal abnormalities including premalignant and malignant lesions. The VELscope illuminates tissue with specific wavelengths that interact with and provide metabolic and biochemical information about the cells at and just beneath the surface. This gives clinicians the ability to see early biochemical changes before they present more obviously, and therefore detect lesions earlier in the disease process. Using history as the gold standard, the device achieved a sensitivity at 98% and specificity of 100% when discriminating normal tissue from severe dysplasia/CIS or invasive carcinoma, according to the BCCA.

This cost-effective, easy-to-use, direct imaging approach allows the dentist, hygienist, and healthcare providers to find lesions that may be hard to see with normal white light, thus improving the quality of care they give to their patients. When the VELscope is used during routine dental examinations, cancer screens can be provided in an efficient way (Figure 2). The procedure takes from 2 to 4 minutes, which makes it easy to incorporate into the usual office protocols (Figures 3 to 6).

Case Reports
The department of Oral Medicine at the University of Washington has, for the past several years, conducted an Oral Dysplasia Clinic that is housed within the Head and Neck Cancer Clinic at the University of Washington Medical Center. It is a regional center that accepts referrals from a 5-state region. The Oral Dysplasia Clinic receives referrals from physicians and dentists treating patients with oral lesions that have or appear to have potential for progression into SCC. The purpose of the clinic is to manage such patients and to follow them periodically to determine if mucosal changes have undergone transformation to aggressive or malignant disease.

The task of periodic evaluation of patients with atypical oral mucosal changes with potential for malignant transformation is difficult and requires judgment calls about the nature of the lesions under observation. Failure to identify malignant transformation effectively can result in a significantly more risky or severe prognosis. Making decisions about whether a lesion represents significant health risk is best described as “making decisions in the presence of uncertainty.” Such situations call for improved methods to assess lesions so that clinical decisions have the highest probability for correctness.

For the past 2 years we have been fortunate to have had access to a prototype VEL-scope unit, and for the past 4 months we have used the newest version of the scope in our clinic. The VELscope has been very helpful in improving our assessment of patients with chronic oral tissue changes and in deciding whether the tissue changes observed using visual inspection are valid or underestimate the risk of malignant disease. The scope does not ensure that all clinical decisions regarding potentially malignant oral lesions are correct, but it has resulted in diagnoses of malignant disease or very aggressive dysplasia in a number of cases that would have otherwise been judged clinically as negative for dangerous change. Among these cases, 4 specifically come to mind that illustrate the value of the VELscope in the assessment and follow-up of patients who present with oral lesions.

**Case No. 1**

A 48-year-old female with no history of tobacco use or other risk factors associated with oral cancer presented to the Oral Dysplasia Clinic upon referral from her head and neck cancer specialist. She had undergone excision of a 4x8-mm inflamed lesion on the right lateral aspect of the tongue that was found microscopically to exhibit moderate to severe dysplasia with carcinoma in situ. At the time of referral she was free of any visually detectable lesion of the oral mucosa. She was seen at 2 follow-up visits 3 months apart, and the tissues of the mouth and tongue remained free of visual changes. At the second follow-up visit she reported mild discomfort in the region of the original lesion excision, and it was thought that her symptom might be a residual neurological effect from the surgery. Clinical inspection failed to identify any tissue change, but careful examination of the right lateral border of the tongue using the VELscope demonstrated a 2x4-mm region of intense light absorption, with the visual appearance of deep purple indicating possible dysplastic or malignant cells.

The cancer surgeon was called to the clinic to observe the region of VELscope-positive tissue change. We recommended biopsy with removal of the area of light absorption along with adequate margins. The biopsy specimen again showed severe oral dysplasia with an area of carcinoma in situ with clear margins. All of the clinical experts who observed the area agreed that had the scope not provided a positive finding, the area would not have been considered for removal, and her symptoms would have been
attributed to postsurgical neurological damage. Her lack of prior risk factors for oral cancer and the absence of a definitive clinical lesion caused us to put dysplasia or carcinoma lower on our list than would otherwise have occurred. The positive findings with VELscope assessment resulted in a decision to biopsy, resulting in discovery of aggressive tissue changes months before they would have become clinically obvious.

Case No. 2

A 62-year-old male with a history of alcohol abuse and heavy use of tobacco for 30 years presented for follow-up of oral lesions on the right posterior of the tongue. Prior biopsy had detected the presence of moderate dysplasia. The patient was followed clinically over a 9-month period at 3 month intervals using very careful visual inspection of the region of prior cellular change. The tissues were palpated, and the patient was cautioned to return earlier than the routine recall date if he noted any tissue change or other symptoms. Clinical inspection of the tongue at each recall visit demonstrated faint leukoplakia that was unchanged in visual appearance or palpation. At the third visit the VELscope examination revealed a 3x4-mm region of dense light absorption in a zone behind the region of leukoplakia. The area was normal to visual inspection using an examination light, and palpation failed to find tactile changes suggestive of thickening of the tissue.

The head and neck surgeon who referred him to the Oral Dysplasia Clinic was informed of the finding and came to the clinic to use the scope to determine the exact region that was VELscope-positive. That area was removed with wide margins, and microscopic examination demonstrated carcinoma with clear margins that did show mild cellular atypia in one region. Use of the scope allowed identification of a region of malignant transformation that was distant from the original site of change and not in the area of leukoplakia that was under close observation.

Case No. 3

A 55-year-old female with a history of SCC of the left posterior maxillary alveolar ridge that had been successfully excised with clear margins was referred to the Oral Dysplasia Clinic for periodic evaluation. She complained of xerostomia and mild oral discomfort that was generalized. Her history was negative for tobacco or alcohol use. Clinical assessment identified a faint generalized mucosal erythema in most areas of the mouth but more dominant on the left than the right, without the presence of any specific oral lesion. The erythema was thought to be associated with the xerostomia, but because of her past history of carcinoma, careful visual inspection of the mucosa along with tissue palpation and VELscope examination was completed. The clinical examination failed to identify specific areas of concern, and no discrete oral lesion was identified. VELscope assessment detected the presence of increased light absorption due to the inflammation in the region, but the combination of VELscope illumination and rough surfaces, tissue friability, and hemorrhage. The past history was positive for regular alcohol consumption for 40 years, mouth, and hard palate. The lesions were a mixture of leukoplakia, erythema, ulceration, and desquamation. Palpation revealed a 4x5-mm region of light absorption (loss of reflectance) that remained after palpation-induced blanching of the surrounding tissue.

The cancer surgeon who had performed her initial surgery was called to observe the VELscope findings, and upon our recommendation the VELscope-positive region was again excised, resulting in a pathologist finding of severe dysplasia and early carcinoma. The presence of generalized erythema likely masked the presence of the lesion, but use of the VELscope allowed identification of premalignant and malignant tissue change from the surrounding erythema associated with the xerostomia. Without the utilization of the scope it is unlikely that the area would have been detected for several months or until it had developed clinical characteristics different enough from the surrounding inflammatory erythema.

Case No. 4

An 86-year-old female of Danish origin presented to the clinic with a complaint of pain and burning of the tissues of the mouth. She had previously been diagnosed, using biopsies, as having erosive oral lichen planus that involved most of the mucosa of the mouth including the buccal mucosa bilaterally, the attached gingiva, the inner surfaces of the lips, all regions of the tongue, the floor of the mouth, and hard palate. The lesions were a mixture of leukoplakia, erythema, ulceration, and desquamation. Palpation revealed rough surfaces, tissue friability, and hemorrhage. Her past history was positive for regular alcohol consumption for 40 years, tobacco use for 30 years, but none for the past 30 years. Her dental status was excellent with 28 intact teeth, but with advancing periodontal disease in the presence of the severe erosive lichen planus of the gingiva. Because of her history she was periodically biopsied, and at one recall visit visual changes on the inner aspect of the lower lip generated a decision to biopsy that area. A wide region of severe dysplasia was detected, and the area underwent laser ablation. She continued to be followed, and with the acquisition of the VELscope it was also employed in her periodic assessments. The areas being watched with particular care were the region of the prior laser ablation margins and the tongue, but at recall it was noted that the erosive lichen planus of the right buccal mucosa had become more severe, so that area was also more intensively evaluated clinically and with use of the VELscope.

The region demonstrated intense VELscope light uptake and was biopsied along with several other areas of the mouth that showed increased erosive activity but not VELscope light uptake. The biopsy of the buccal mucosa revealed moderately well-differentiated carcinoma, even though it did not clinically look different than other regions of erosion from lichen planus. Use of the scope allowed us to detect malignant change in the field of tissue changes that were not malignant at the time and to target our observations more carefully. Since the original VELscope-positive finding, we have observed another region positive for VELscope light absorption that also demonstrated severe dysplasia and early carcinoma. The patient continues to be followed at 2-month intervals.

Summary

The VELscope is an important aid in patient assessment, and when added to a well-thought-out clinical assessment process that takes into consideration the age of the patient and risk factors that include tobacco, alcohol, and immunologic status, it increases the clinician’s ability to detect oral changes that may represent premalignant or malignant cellular transformation. False positive
findings are possible in the presence of highly inflamed lesions, and it is possible that use of the scope alone may result in failure to detect regions of dysplasia, but it has been our experience that use of the VELscope improves clinical decision making about the nature of oral lesions and aids in decisions to biopsy regions of concern. Where tissue changes are generalized or cover significant areas of the mouth, use of the scope has allowed us to identify the best region for biopsy. As with all clinical diagnostic activities, no single system or process is enough, and all clinicians are advised to use good clinical practice to assess patients and to recall and biopsy lesions that do not resolve within a predetermined time frame. Lesions that are VELscope-positive and absorb light need to be followed with particular caution, and if they do not resolve within a 2-week period, then further assessment and biopsy are generally advised. It is much better to occasionally sample tissue that turns out to be benign than to fail to diagnose dysplastic or malignant lesions.

In our fight to protect patients from cancer, the VELscope improves our odds for early detection, hopefully resulting in fewer deaths from oral cancer.

References


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Fluorescence Visualization Detection of Field Alterations in Tumor Margins of Oral Cancer Patients

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Abstract

Purpose: Genetically altered cells could become widespread across the epithelium of patients with oral cancer, often in clinically and histologically normal tissue, and contribute to recurrent disease. Molecular approaches have begun to yield information on cancer/risk fields; tissue optics could further extend our understanding of alteration to phenotype as a result of molecular change.

Experimental Design: We used a simple hand-held device in the operating room to directly visualize subclinical field changes around oral cancers, documenting alteration to fluorescence. A total of 122 oral mucosa biopsies were obtained from 20 surgical specimens with each biopsy being assessed for location, fluorescence visualization (FV) status, histology, and loss of heterozygosity (LOH; 10 markers on three regions: 3p14, 9p21, and 17p13).

Results: All tumors showed FV loss (FVL). For 19 of the 20 tumors, the loss extended in at least one direction beyond the clinically visible tumor, with the extension varying from 4 to 25 mm. Thirty-two of 36 FVL biopsies showed histologic change (including 7 squamous cell carcinoma/carcinomas in situ, 10 severe dysplasias, and 15 mild/moderate dysplasias) compared with 1 of the 66 FV retained (FVR) biopsies. Molecular analysis on margins with low-grade or no dysplasia showed a significant association of LOH in FVL biopsies, with LOH at 3p and/or 9p (previously associated with local tumor recurrence) present in 12 of 19 FVL biopsies compared with 3 of 13 FVR biopsies ($P = 0.04$).

Conclusions: These data have, for the first time, shown that direct FV can identify subclinical high-risk fields with cancerous and precancerous changes in the operating room setting.

In 1953, Slaughter published a hallmark article in which he emphasized the importance of examining the field surrounding oral cancers for both risk assessment and management of this disease (1). There has been extensive research in this area since then, more recently, using molecular technology. It is becoming increasingly apparent that genetically altered cells could become widespread across the epithelium of patients with oral cancer, into clinically and histologically normal tissue, and that these cells could drive the process of field cancerization (2, 3). In recognition of this, surgeons try to remove oral squamous cell carcinomas (SCC) with a significant width of surrounding normal-looking oral mucosa, if anatomically allowed. However, the occult disease varies in size and a wealth of evidence suggests that it frequently extends beyond the tumor clearance. This extension may be responsible for the high rate of recurrence of carcinomas at the primary site (~10-30% of cases; refs. 4–9). There is a pressing need to develop new approaches that can be easily used in clinical practice to facilitate the detection of these clinically occult fields.

One such new approach may involve the use of tissue optics. The association of cancer development with the loss of normal tissue autofluorescence has been reported for a number of tissues and organs (10–15). More recently, visual aids using optical methods to detect such loss have been shown to reveal premalignant and malignant lesions that are not detected by unaided eyes (16–18). We have reported the development of a simple hand-held device that facilitated the detection of autofluorescence loss in both visible and occult high-risk oral lesions through direct fluorescence visualization (FV; refs. 17, 18). The interaction of light with tissue has generally been found to highlight changes in the structure and metabolic activity of the areas optically sampled. Specifically, the loss of autofluorescence is believed to reflect a complex mixture of alterations to intrinsic tissue fluorophore distribution, such as the breakdown of the collagen matrix and a decrease in flavin adenine dinucleotide concentration due to tissue remodeling and increased metabolism associated with neoplastic development. Correspondingly, structural changes in tissue morphology associated with neoplastic development...
in both the epithelium and lamina propria (e.g., thickening of the epithelium, hyperchromatism and increased cellular/ nuclear pleomorphism, or increased microvascularity), lead to increased absorption and/or scattering of light, which in turn, reduces and modifies the detectable autofluorescence (16, 17, 19, 20).

The objective of this study was to investigate the value of this device in the operating room to delineate field change in autofluorescence around cancers by determining and comparing the histopathologic and molecular changes of margin biopsies that retained normal FV with those margin biopsies that showed a loss of FV. We chose microsatellite analysis for loss of heterozygosity (LOH) at 3p, 9p, and 17p as the molecular analysis, a method used by many international groups to mark clonal spread and possibly predict recurrence (21). A recent study showed that detection of LOH at 3p and/or 9p at prior cancer sites (after tumor removal) was strongly associated with tumor recurrence: samples with such loss had a 26.3-fold increase in the risk of developing second oral malignancy at the site compared with those that retained both of these arms (22). This current study showed a frequent loss of FV of varying distances (up to 25 mm) in clinically normal–looking mucosa surrounding the tumors and a strong concordance between loss of autofluorescence in tumor margins and the presence of significant histologic change and molecular risk.

**Materials and Methods**

**Patients.** Twenty consecutive patients with biopsy-confirmed primary cancer of the oral cavity were accrued to the study as they presented at the British Columbia Cancer Agency between July 2004 and February 2005. Eligibility criteria included the presence of early stage disease (T0-T2) scheduled for surgical excision with intent to cure. All the patients were >18 years of age and provided informed consent. Of the 20 cases in this study, 65% were male, 65% had a smoking history, and 75% were Caucasian. The average age was 58 (36-80 years). Tumor staging was determined from surgical specimens using American Joint Committee on Cancer Staging criteria (23): eight carcinomas in situ (CIS, stage 0) and five stage I and seven stage II invasive SCCs (Table 1). Nine of the SCCs were well to moderately well differentiated with the remaining three poorly differentiated. The majority of the tumors were from the tongue (17 of 20, 85%), with one case from the floor of the mouth, and two from the gum.

**The FV device.** A description of the research FV device and its use is given in Lane et al. (17). Briefly, it consists of a bench-top light source coupled to a hand-held unit for direct visualization. Lesions were

<table>
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<tr>
<th><strong>Patient ID</strong></th>
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<th><strong>Demographics</strong></th>
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<sup>a</sup>Histology: Cancer (light blue), high-grade dysplasia (red), low-grade dysplasia (yellow), no dysplasia or cancer (green), not applicable (gray), no extension of FVL beyond clinical tumor.

<sup>c</sup>Distance: values reflect the distance from the boundary of clinically apparent tumor to the FVL boundary (mm).
illuminated by this blue/violet light source and then directly visualized through long pass and notch filters, which allow the passage of green and red autofluorescence.

Under direct FV, the normal oral mucosa emits various shades of pale green autofluorescence. Clinical lesions that retained the normal green autofluorescence under FV were defined as FV retained (FVR). Tissue which showed a reduction in the normal pale green and appeared as dark patches were classified as FV loss (FVL; see example in Fig. 1C; ref. 18). This distinction involved a comparison of the lesion site with both adjacent tissue and, as an anatomic control, with tissue on the contralateral side.

Photographs of tissue fluorescence were acquired using illumination from the FV device and a digital single lens reflex camera (Fuji FinePix S2 Pro, Fujifilm, Odawara, Japan) with a long-pass filter (Schott GG475-3, Howard Glass, Worcester, MA). The single lens reflex camera was equipped with a 105 mm f/2.8 macro lens (Nikkor-Micro, Nikon, Tokyo, Japan) and a ring flash (Nikon Macro Speedlight SB-29s, Tokyo, Japan) for white-light images.

Surgical field assessment of FV status. The protocol involved the examination of the surgical site of each patient under both regular operating room illumination and with direct FV, in a stepwise fashion as shown in Fig. 1. All procedures were done while the patient was under general anesthesia and each step was photographed for documentation. The steps included an initial assessment under regular operating room light (Fig. 1A, step 1), demarcation of the boundary of the clinical tumor using a blue marker (Devon skin marker, Ludlow Company, Chicopee, MA) as judged by the surgeon (D.W. Anderson or J.S. Durham; Fig. 1B, step 2), followed by assessment of the site for altered fluorescence using direct FV (Fig. 1C, step 3). The latter examination was done with the light turned off, using the FV device. Areas showing loss of normal green fluorescence were outlined, demarcating FVL boundaries (Sharpie green marker, Sanford, Oak Brook, IL; Fig. 1D, step 4). Then the light was turned back on, the distances between the clinically visible tumor under white light and FVL boundaries were ascertained using a flexible ruler (Devon skin marker, Ludlow) in four directions: anterior, posterior, medial (to the sagittal plane or dorsum tongue), and lateral (to the sagittal plane or floor of mouth margin). Finally, an electroknife was used to outline the surgical boundary (Fig. 1E, step 5).

Tissue sampling and histologic assessment. After resection, a total of 122 punch biopsies (5 mm) were taken from the tumor and from the tumor margins with at least one margin biopsy from each of the four directions (Fig. 1F, step 6 and Fig. 2). All biopsies were fixed in formalin and submitted for histopathologic evaluation by study pathologists without knowledge of FV status (L. Zhang, R.W. Priddy, and K.W. Berean).

Microsatellite analysis of tumor margins. All FVL biopsies from the tumor margins with a histologic diagnosis of low-grade dysplasia (15 biopsies) or no dysplasia (4 biopsies) were microdissected and analyzed for LOH (Fig. 2, see LOH analysis). As a control, an additional 13 biopsies were analyzed from FVR margins. The protocols for digestion and extraction of samples, LOH analysis, and scoring are described in Zhang et al. (13). All samples were coded so that LOH analysis was done without knowledge of diagnosis or FV status. Microsatellite markers that were used mapped to the following 10 regions: 3p14.2 (D3S1234, D3S1228, and D3S1300), 9p21

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**Fig. 1.** Stepwise protocol used for assessing surgical field. A, in the operating room, initial assessment under white light of an ill-defined SCC at right ventrolateral tongue; B, clinically apparent tumor outlined in blue; C, assessment of field using FV in the dark; D, FVL area outlined in green in the dark; E, boundary of surgical specimen (red); F, blocking of surgical specimen, showing location of punch biopsy sites from clinically visible tumor (red circle), from tissue showing FVL, placed directly abutting FVL boundary (green circle), and, from tissue showing FVR, placed directly abutting the boundary of surgical specimen (blue circle).
A total of 122 oral mucosa biopsies were obtained from the 20 tumors, 20 from the clinical tumor itself and 102 from the tumor margins. Figure 2 shows the study design and summarizes biopsy-specific data obtained for location, FV status, histology, and LOH. For each surgical sample, there were three boundaries: the boundary of the clinically apparent tumor (Fig. 1B), the FVL boundary (Fig. 1D), and the surgical boundary (Fig. 1E). Thirty-six margin biopsies were obtained from FVL tissue and these were placed adjacent to the FVL boundary. The 66 FVR margin biopsies were placed adjacent to the surgical boundary (Fig. 1F).

Novel FVL fields extend beyond the clinical boundary. All tumors showed a loss of fluorescence (FVL), regardless of tumor stage and grade of differentiation. In 19 of 20 tumors, FVL boundaries extended beyond the clinically apparent lesion (Table 1). The extent of this subclinical FVL extension varied considerably, ranging from 4 to 25 mm (mean, 10.3 ± 5.7 mm), with 10 tumors showing a >10-mm FVL extension in one or more directions. It is important to note that FVL extension was never evenly distributed around any given tumor. For example, the tumor in Fig. 1 showed subclinical FVL extension primarily in the posterior direction; in contrast, most of the extension in the tumor in Fig. 3 was in the anterior and lateral directions, with minimal extension in the medial and posterior directions.

To investigate the possibility that the advent of invasion is accompanied by a more aggressive lateral/horizontal subclinical FVL spread, we compared the margin mapping data in the 8 preinvasive high-grade lesions (CIS) with the 12 invasive SCCs. The average width for subclinical FVL extension beyond the clinical boundary was similar for CIS and invasive SCCs (10.4 ± 6.7 versus 10.2 ± 5.6 mm; P = 0.79).

FV identifies the majority of histologic risks. As shown in Fig. 2, among the 36 FVL margins, there were 7 (19%) cancers (CIS/SCC), 10 (28%) high-grade dysplasias, 15 (42%) low-grade dysplasias, and 4 (11%) cases with no dysplasia. In contrast, only 1 of the 66 FVR margins was dysplastic. In other words, FVL identified 32 of the 33 cancerous or dysplastic biopsies in the 102 margin biopsies, including all of the cancerous and high-grade dysplasias. There was a significant correlation between the presence of high-grade dysplasia and above with loss of FV (P < 0.0001).

Of the 10 tumors showing >10-mm FVL extension at one or several directions of the tumor margins, 6 tumors showed histologic changes of high-grade dysplasia and above in biopsies taken from FVL regions >10 mm from the clinical tumor boundaries (Table 1: cases 1, 12, 14, 15, 17, and 19).

Molecular risk assessment of low-grade lesions. Because histology is a poor indicator of outcome for margins with little (low-grade) or no histologic change, we used molecular analysis to further define risk for FVL and FVR margins. An example of this combined analysis and its value in assessing FVL margins is shown for case 10 (Fig. 3).

Microsatellite analysis of LOH at 3p, 9p, and 17p was done for 32 biopsies, consisting of all 19 FVL margins showing low-grade dysplasia or no dysplasia, and 13 FVR margin biopsies: the single case with mild dysplasia and 12 randomly chosen cases with no dysplasia (Fig. 2). As shown in Fig. 4A, consistently higher rates of LOH in all categories of comparisons were observed in FVL margins as compared with FVR margins. Such higher rates were significant at 9p (53% versus 8%, P = 0.01), for >1 arm lost (37% versus 0%, P = 0.03), for LOH at 3p and/or 9p only (63% versus 23%, P = 0.04), and for 3p and/or 9p plus 17p (37% versus 0%, P = 0.03; Table 2). Strikingly, of the four FVL margins with no dysplasia, two showed LOH at 3p and/or 9p plus 17p, and one showed LOH at 3p (Fig. 2). Of the 13 FVR margins, 3 also showed LOH at 3p and/or 9p, including the single mild dysplasia that was FVR.

As mentioned above, six tumors showed histologic changes of high-grade dysplasia and above in biopsies taken from FVL regions >10 mm from the clinical tumor boundaries. Molecular assessment showed an additional two cases with molecular risk in biopsies taken from FVL regions >10 mm from the clinical tumor boundaries (Table 1: cases 3 and 6).
Molecular technology has begun to shed new light on the definition of “field-at-risk” in patients with oral cancer. In this study of fluorescence field changes, we show that the development of new optical techniques that enable us to visualize spectral alterations associated with oral cancer could add a further dimension to these developing paradigms regarding the concept of cancer/risk field.

Our data indicate strongly that the field of FV alterations (FVL) within or beyond the clinically apparent tumor area is associated with morphologic high-grade and molecular high-risk tissue change. All the 20 tumors in this study displayed FVL. All but 1 of the 36 margin biopsies from the subclinical FVL field had either histologic dysplasia/cancer and/or genetic alterations associated with molecular risk. Seventeen of the 36 cases (47%) had cancer or severe dysplasia and 15 cases (42%) had low-grade dysplasia. Nine of the 15 latter cases showed LOH at 3p and/or 9p, a molecular pattern associated with a 26-fold increase in relative cancer risk for tumor recurrence (29). Only 4 of the 36 (11%) FVL margins were not dysplastic; however, three of the four biopsies showed LOH at 3p and/or 9p when assessed molecularly. In contrast, only 1 of the 66 FVR margins was dysplastic (low-grade) and 3 of the 13 FVR margins analyzed for LOH showed molecular risk (includes the dysplastic case).

These findings add to the growing evidence that supports the use of FV to detect cancers and high-risk lesions (16, 30–32), including occult or nonapparent lesions/areas (18). The closest report existing in the literature to our present study is that of Svistun et al. (16) in which the authors evaluate a similar visual analysis system on excised oral cancer tissue and surrounding tissue ex vivo. The best subset of the illumination and detection wavelengths found in their study is identical to the ones used by the FV device in the present study. Although they had a small number of cases (four), their limited results indicated a correspondence between pathology and abnormal fluorescence.

A limitation of the study, however, was the use of excised tissue and the identification of areas of altered fluorescence by a surgeon using pictures of this tissue under different conditions.

One of the most difficult and contentious issues with respect to treatment of oral cancers involves the decision on the width of clinically normal tissue that should be removed in addition to the tumor. In an effort to remove occult high-risk field change, surgeons frequently remove an arbitrary 10 mm or more of normal-looking mucosal margin when excising oral cancer, if anatomically possible. Unfortunately, this approach still fails to completely remove the occult high-risk field changes in many patients, resulting in a high-rate of tumor recurrence. Our data showed that such occult change is a frequent event (found in 19 of the 20 tumors), and that the width of this subclinical extension varies considerably (4-25 mm), frequently extending in at least one direction by...
and molecular techniques could complement each other. For example, surgical margins of oral cancer have been examined intraoperatively using quantitative methylation-specific PCR and methylation-positive margins have been identified (33). Optical devices could enhance this molecular mapping. In turn, the assessment of FV boundaries for such molecular change or others (e.g., p53 mutation with mutation-specific plaque hybridization assay; ref. 5) would improve our understanding of the nature of this new phenotype. It should be noted that the need for multiparameter assessment of the cancer field also includes the development of new approaches to assessing the depth of cancer extension in vivo, as the current device assesses mainly lateral cancer spread.

Finally, our data found no difference between CIS and invasive SCC in terms of the FV field expansion. The information is important because the usual recommendation for preinvasive high-grade lesions tend to be more conservative with smaller margins of normal-looking mucosa. The study results suggest that a subgroup of these preinvasive lesions may have extensive lateral fields, some occult, and as such, would require a more aggressive therapy.

In summary, the current study is an important step in the development of a potential integration of optical technology into the management of patients with oral cancer. The device will need to be integrated with information from other sources, both histologic and molecular, and experience with the device will have to be associated with clinical outcome before its clinical value can be established. However, as a proof-of-principle, our data has, for the first time, shown that direct FV can identify subclinical high-risk fields with cancerous and precancerous changes in the operating room setting.
Oral Cancer Screening in High-risk Underserved, P.1

**Oral Cancer Screening in the Vancouver Downtown Eastside -- a High-Risk Underserved Population**

**Running Title:** Oral Screening in High-Risk Underserved

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ABSTRACT

Background: With the advent of new technology and a better understanding of the natural history of oral cancer, there is increasing pressure to develop screening strategies for its prevention and early detection. This need is even more imperative among marginalized high-risk communities. The objective of this paper is to describe our first year’s experience in a screening clinic using innovative visualization aids in the Vancouver Downtown Eastside (DTES).

Methods: Demographics, risk factors and medical history were collected by personal interview from patients seen at the Portland Community Dental Clinic located in the Vancouver DTES. Patients received a full head and neck examination including the use of novel visualization aids (fluorescence visualization and toluidine blue stain).

Results: Of 133 patients examined, the majority were at high-risk: ever smokers (86%) and regular consumers of alcohol (83%). On clinical examination, 26 had clinical leukoplakia. All 26 showed clinically significant alteration in fluorescence at the lesion site, with 31% also showing retention of toluidine blue. To date, 6 have been biopsied, showing 1 case with oral cancer and 4 with oral premalignant dysplasia.

Conclusion: We were able to recruit patients from Vancouver DTES, a poor, medically underserved population, and were able to identify cases with significant disease that required further triage for treatment. Challenges and possible solutions with recruitment for screening and subsequent treatment are discussed.

Keywords: Oral cancer screening, High-risk, Underserved, Visualization aid.
INTRODUCTION

Oral cancer, a leading cancer in many parts of the world, occurs in 3,100 Canadians and is responsible for 1,050 deaths each year. It has one of the worst prognoses among major human cancers (< 50% five-year survival rate), which is largely because many of these lesions are diagnosed late (stage III or IV cancers). Key to improving this outcome is the detection of the disease at an earlier stage. This is hampered by the lack of devices/tools that facilitate the identification of early disease, and equally important, an inability to reach high-risk populations with these devices.

Several investigators have reported benefit from oral cancer screening. Sankaranarayanan et al recently showed that periodic examination of the oral cavity can reduce mortality from oral cancer in high-risk individuals in India. These findings are relevant to Canada because, amazingly, 42% of the newly diagnosed oral cancer in British Columbia are late-stage.

One limitation to oral cancer screening is the lack of effective tools for early detection. There has been a recent evolution of visualization aids that appear to enhance the detection of high-risk oral premalignant lesions and cancer. We have shown that fluorescence visualization can identify the majority of premalignant dysplastic lesions, including those that are clinically occult. In addition, retention of toluidine blue identifies oral premalignant lesions at markedly increased risk of developing into oral cancer. The latter work was conducted in referral clinics among cases with known
dysplasia. The current paper describes the introduction of these screening techniques into a community clinic serving a hard-to-reach and high-risk population.

Persons at high-risk for oral cancer are often difficult to reach for early detection and subsequent treatment. One such population in Vancouver is the Downtown Eastside (DTES); its residents are among Canada’s poorest residents from diverse backgrounds. This is a high-risk population for oral cancer because many are heavy smokers and alcohol drinkers, the main risk factors for oral cancers\textsuperscript{12} and are rarely seen by physicians or dentists. A unique opportunity arose to reach this population through the Portland Community Dental Clinic, which provides quality dental care to residents of DTES. The purpose of this paper is to describe our first year of experience working in this community.

METHOD

Study Group

In September 2004, an Oral Cancer Screening Clinic was established within the Portland Community Dental Clinic, involving collaboration between dental staffs (two staff dentists, a dental hygienist, a receptionist, and two certified dental assistants) and the multi-disciplinary research team (an oral medicine specialist, two master students, and a research member). The Clinic is located within one of the 6 hotels within the Portland Hotel Society, which provides housing for approximately 600 DTES residents (see Figure 1).

Eligibility for this study included current residency in the DTES, mental competency, attendance at the Portland Community Dental Clinic, and consent to receive
an oral cancer screening examination between September 2004 and July 2005. The findings reported in this paper refer to the initial visit for these participants. The study protocol was approved by the University of British Columbia Research Ethics Board and all patients signed informed consent before enrollment into the study.

Data collection - Data were collected on demographics, risk factors and medical history by personal interview. Each patient then received a full head and neck examination. Fluorescence visualization (VELscope, LED Medical Diagnostics Inc., White Rock, BC) was done on all participants to assess the entire oral cavity. Clinically suspect lesions identified by the oral medicine specialist (CP) were further assessed for retention of toluidine blue stain. Clinical findings were recorded as part of the dental record. Oral brushings were done to collect exfoliated cells and these samples were archived in liquid nitrogen for future analysis. Descriptive statistics were used to summarize the findings.

RESULTS

Study recruitment – One hundred and thirty-three patients were screened in 28 sessions over the study period. All participants completed the personal interview and head and neck examination, including VELscope intraoral screening. Twenty-six patients were identified by the oral medicine specialist to have clinically suspicious lesions and all were assessed by toluidine blue staining.

Demographics - As seen in Table 1, the recruited patients were mostly male (77%), white (76%), and middle-aged (average age, 47.3 years). They represented an underserved population, being largely unemployed (78%), on low-income [83%,
<$12,000 annually: on welfare (47%) or disability benefits (35%)], and with less than post-secondary education (58%). Among these cases, 10% were First Nations and 3% were Chinese.

**Risk factors** - The recruited patients were also at increased risk for oral cancer, being predominantly ever smokers (86%) and regular consumers of alcohol (83%). A sizeable proportion reported being immuno-challenged (39%). Drug use was common, with 42% of individuals on antidepressants or anti-psychotics, 8% on methadone therapy and 51% on street drugs (crack/cocaine, heroine and/or crystal meth). Of interest, only 7 of 15 HIV patients were currently receiving anti-retroviral therapy. Seven percent reported a history of head and neck squamous cell carcinoma in immediate family members.

**Reason for Visit** – Most patients presented with dental needs, these most commonly being tooth-related (50%), for dentures (24%) and for hygiene (18%) (see Table 1). Only 2 patients were referred for oral cancer screening.

**Clinical Findings** – Seventy-eight patients (59%) showed a clinical anomaly requiring follow-up, including the presence of clinical leukoplakia (20%), traumatic lesions requiring a further visit to rule out premalignancy (24%) and oral candidiasis (10%). All patients with clinical leukoplakia were examined for fluorescence visualization alteration and retention of toluidine blue (Figure 2). All clinical leukoplakia showed clinically significant alteration in fluorescence and 8 patients (31%) showed retention of toluidine blue. As of September 2005, 6 of the 26 patients with clinical leukoplakia have been biopsied. Of these, 1 was histologically confirmed to be cancer, 4 to be dysplasia and 1
showed chronic hyperplastic candidiasis with basilar atypia requiring re-assessment after antifungal therapy for possible premalignancy.

DISCUSSION

Reaching marginalized individuals is a major challenge in all screening programs. Although a growing literature has focused on barriers to screening, to our knowledge there are no functioning oral cancer screening clinics in high-risk communities in Canada.

In Vancouver, a particularly high-risk and marginalized community resides in the Downtown Eastside, who are among Canada’s poorest residents from diverse backgrounds. This population of approximately 16,000 individuals is at high-risk for oral cancer because many are heavy smokers and regular consumers of alcohol, with often a history of substance abuse. Further aggravating the problem is that many are immunocompromised, frequently having poor nutrition and oral hygiene, and lacking dental and medical care.

In the first year of our Oral Cancer Screening Clinic, we were able to reach only a small proportion of the resident population. However, among the first 133 participants, we detected 1 individual with oral cancer (stage II or T2) and 4 with premalignant dysplasia. This is a very high incidence of disease given that the national average incidence for oral cancer is only 1 in 10,000.1

One of the main outcomes of this study was that it yielded information on the challenges of working with this community. Firstly, all patients attending the Portland Community Dental Clinic were either scheduled or emergency cases and, of these, only
70% received a screening examination. We were unable to screen the remaining patients for a variety of reasons including extensive dental treatment, severe psychological problems, and/or current level of drug or alcohol intoxication which would make it inappropriate to conduct the screening examination. In addition, some patients refused to participate because they stated having a lack of time. Secondly, although this is a small neighbourhood, residents are hesitant to move outside very small boundaries. In fact, only 7 patients from Portland Hotels attended the Clinic (see Figure 1). Many residents have no fixed address and patients were often referred to the Clinic by a street nurse.

This territorial finding may be attributed to a variety of reasons, including safety issues and the distribution of ethnic groups within the neighbourhood. The majority of participants screened were Caucasian, with only 10% First Nations and 3% Chinese. According to census data, the DTES is a very ethnically diverse population with 33% Chinese.13 There was also gender imbalance in that only 23% of the participants were female as compared to 33% in the DTES population.13

We are exploring possible solutions to these challenges. To guide these initiatives, several focus groups of DTES residents are being conducted, including both persons who attended, and not attended, the screening clinic. We are also increasing dialogue with community leaders among the Portland Hotel Society, the First Nations and the Chinese communities. To address potential gender imbalance, oral cancer screening is now being offered during monthly women’s health days, a program already established within the Portland Community Dental Clinic.

We have also identified a challenge in the management of participants with abnormal screening results. Twenty-six patients with clinical leukoplakia were
identified; however only 6 have been biopsied to date. This is due in part to difficulty in getting patients to return for follow-up, since safety and poverty issues are often given a higher priority. It is difficult to contact some participants because of no fixed address. One possible solution would be to provide a biopsy service at the Clinic, rather than refer patients to another location for follow-up. This action could then allow for immediate biopsy at the time of screening.

The management of high-risk disease is another issue that needs to be addressed in the development of a comprehensive strategy for control of oral cancer in this population. We are in the process of developing a fast-track triage-to-treatment pathway for high-risk patients in British Columbia.

In closing, we have positioned an oral cancer screening clinic within a previously existing dental clinic serving a high-risk and hard-to-reach community and have demonstrated both the need for this service and the ability to identify high-risk individuals. The work was done by a highly skilled team, with an oral medicine specialist and support staff trained in the use of state-of-the-art visualization tools. British Columbia is pioneering in the development of a province-wide prevention program, the BC Oral Cancer Prevention Program, to integrate risk identification and management strategies within a cost-effective framework. Key to this development is to reach the high-risk and underserved populations.
REFERENCE


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Table I
Demographic characteristics, risk factors and clinical findings

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* SCC, Squamous Cell Carcinoma
† Immune status: compromised (HIV infection), complicated (Hepatitis C infection, Hepatitis B Carrier or Diabetes)
Figure Legends:

Figure 1. Map of the Vancouver Downtown Eastside. ●, indicates the location of Portland Hotels; and ★, indicates location of the Portland Community Dental Clinic. The insert map shows the location of the DTES within the City of Vancouver.

Figure 2. New tools to identify suspect clinical change in a DTES patient. A, An ill-defined red lesion on the left buccal mucosa of a 65 year-old male. B, The same lesion, viewed with a VELscope, showing a well-demarcated dark area of fluorescence visualization loss. C, Lesion after application of vital stain toluidine blue (TB) showing focal uptake of the blue stain. Biopsy from site with TB staining showed an invasive squamous cell carcinoma.
Figure 1.

![Map of Downtown Eastside Communities](image)

Figure 2

A  
B  
C
CASE REPORT

Dennis H. Kraus, MD, Section Editor

DIRECT FLUORESCENCE VISUALIZATION OF CLINICALLY OCCULT HIGH-RISK ORAL PREMALIGNANT DISEASE USING A SIMPLE HAND-HELD DEVICE

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Abstract: Background. A considerable proportion of oral cancer and precancer is not clinically apparent and could contribute significantly to the late diagnosis and high mortality of oral cancer. A simple method to identify such occult change is needed.

Methods. Patients in the Oral Dysplasia Clinics at British Columbia are currently being examined with a simple hand-held device that permits the direct visualization of alterations to autofluorescence in the oral cavity. Tissue showing loss of autofluorescence is biopsied.

Results. We present 3 representative cases in which occult lesions were identified with fluorescence visualization during longitudinal follow-up, resulting in the diagnosis of a primary dysplasia in case 1, a second primary cancer in case 2, and cancer recurrence in case 3.

Conclusions. This is the first report of the diagnosis of occult oral disease using a simple noninvasive device. These early examples indicate the potential value of this technology to guide the management of patients with oral lesions, facilitating the detection of high-risk changes not apparent with white-light visualization. © 2006 Wiley Periodicals, Inc. Head Neck 29: 71–76, 2007

Keywords: oral premalignant lesion; oral cancer; autofluorescence; fluorescence visualization; early detection

A consensus is gradually developing that there is a need to broaden the focus in patients with oral cancer and precancer beyond the clinical lesion, placing greater emphasis on such patients as having a high-risk condition or disease. Clinically visible oral lesions may represent the “tip of the iceberg,” signaling the presence of multiple or widespread subclinical changes to the tissue.1 New technology needs to be developed to identify such subclinical changes early to improve prognosis.

Direct fluorescence visualization is 1 potentially powerful approach that may be used routinely by clinicians in the future to facilitate the visualization and management of nonapparent
lesions. Ultraviolet light has been used for disease detection since the 1950s. The goal of these devices has always been to enhance the visualization process. Visualization under standard room light can only perceive a fraction of the spectral differences that exist between diseased and normal tissue that optical techniques, especially those based on fluorescence imaging, may reveal. Data are accumulating from both laboratory and clinical studies that suggest that changes in natural fluorescence reflect biochemical and morphological alterations to tissues that could serve as noninvasive indicators of high-risk lesions.

In this study, we present early research done using a simple hand-held device (Figure 1) for the direct visualization of tissue fluorescence alteration in the oral cavity. This device illuminates the oral mucosa, exciting natural fluorophores in the tissue and causing them to emit fluorescence that is visualized directly by a human observer.

In a pilot study of 44 patients, we have shown that the device achieved a sensitivity of 98% and specificity of 100% when discriminating normal mucosa from severe dysplasia/carcinoma in situ (CIS) or invasive carcinoma. In this study, we report on our experience using this device to assess patients entering longitudinal follow-up at the Oral Dysplasia Clinics in British Columbia. We present 3 representative cases in which occult lesions were identified with fluorescence visualization, resulting in the diagnosis of a primary dysplasia in a noncancer patient (case 1), a second primary cancer in a patient with a history of oral cancer (case 2), and cancer recurrence in the third patient (case 3).

CASE REPORT

Case 1 involved a 51-year-old white woman who had no history of tobacco use. In November 2003, a severe dysplasia was excised by laser from her left ventral tongue. In February 2005, oral examination at the Dysplasia Clinic in the British Columbia Cancer Agency (BCCA) showed no apparent oral lesion at the previous surgical site under white light examination (Figure 2A). Strikingly, fluorescence visualization examination detected a large area showing loss of autofluorescence (termed FVL for fluorescence visualization loss) posterior to the previous surgical site (Figure 2B). The FVL site appeared dark green to black. In contrast, the surrounding oral mucosa, as an internal anatomic control showed normal pale green autofluorescence (termed FVR for fluorescence visualization retained). A comparative follow-up biopsy of the FVL area showed a moderate epithelial dysplasia (Figure 2C).

Case 2 involved a 43-year-old white female smoker who had a CIS removed surgically from the left floor of mouth in October 2002. At the 1-year recall examination (October 2003), the operating ear-nose-throat (ENT) surgeon reported her examination unremarkable. The patient was also seen by the oral maxillofacial medicine specialists at the Oral Dysplasia Clinic the same day. Again, a scar at the former cancer site was observed but no apparent oral lesion under white light. In contrast, fluorescence visualization examination detected a well-demarcated area of FVL at the right lingual aspect of retromolar and soft palate region (Figure 2E). A mild erythematous area was then noted on reexamination (Figure 2D).

FIGURE 1. Illustration of direct fluorescence visualization technique. The procedure uses a hand-held viewing instrument (A) connected to a light source, which is used to illuminate the target tissue with an excitation light (extracoral light source) that is blue in color (400–460 nm). The target tissue fluoresces (green-red) under the excitation light, and the light produced by the tissue is called autofluorescence. An emission filter blocks the blue excitation and ensures that only green and red light is transmitted for direct visualization to the operator (B). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
This area had no previous biopsy or treatment. Incisional biopsy showed a CIS (Figure 2F), which was subsequently removed completely. Recurrence has not been seen at last follow-up 19 months later in May 2005.

Case 3 involved a 56-year-old Asian male smoker who had a CIS removed from his left ventral tongue by laser in July 2002. At the 18-month recall examination (January 2004), a slightly diffuse, erythematous change was noted at the previous surgical site (Figure 3A). Both the operating ENT surgeon and an oral maxillofacial medicine specialist at the Dysplasia Clinic regarded this as within the normal limit of postlaser treatment mucosal alteration. Fluorescence visualization examination, however, detected a very large, well-demarcated area of FVL, close to 4 cm in size (Figure 3B). Three punch biopsies were taken: 1 from the anterior orange-colored FVL area (arrow head) showing severe dysplasia (Figure 3D), a second from the posterior dark-colored FVL area (arrow) showing CIS (Figure 3E), and a final biopsy from an FVR area (asterisk) immediately anterior to the orange-colored FVL site, which was shown to be unremarkable histologically (Figure 3C).

There was an obvious layer of bacteria on the epithelium surface in D, which was probably responsible for the perceived orange fluorescence at this site. Studies have shown that orange auto-

FIGURE 2. Detection of clinically occult disease in cases 1 (left panel) and 2 (right panel) using direct fluorescence visualization. Case 1 shows an occult lesion at the left ventral tongue under white light examination (A), which is identified by fluorescence visualization (B, arrow). The associated histology (C) shows moderate dysplasia with thickening of the epithelium and subepithelial connective tissue with a mild degree of inflammatory cell infiltration and increased capillaries. Case 2 shows a nonapparent carcinoma in situ (CIS) at right lingual retromolar and posterior soft palate region under white light (D), which was identified by fluorescence visualization (E, arrow). Biopsy revealed a CIS (F) with marked epithelial thickening with areas of atrophy. The connective tissue showed a heavy inflammation with increased vasculature (C and F: hematoxylin and eosin stain, original magnification: ×100).
fluorescence is associated with bacterial infection/host response as a result of bacterial porphyrins. High-risk lesions could demonstrate perceived orange autofluorescence, because the signal is a mixture of the tissue autofluorescence and the bacterial autofluorescence due to subclinical infection. However, the significance of orange autofluorescence should be assessed in conjunction with other clinical factors. If its presence could be explained by mucosa infection or its appearance is on dorsal tongue where there is frequent bacterial lodging, the orange fluorescence does not indicate risk.

The large field was removed again by laser. Recurrence was not seen at last follow-up 23 months later in December 2005.

DISCUSSION

There is a growing awareness that potentially malignant intraoral lesions and early cancers do not always manifest clinically. Our current inability to detect many of these early changes clinically could contribute significantly to the late diagnosis of this disease and its dismal prognosis, even though the oral cavity is a site that is readily available for assessment.

There is an increasing interest in using optical technology to provide a more complete picture of the alterations that occur during the development of cancer, identifying alterations to biochemistry and morphology that lie beyond the visual range. Autofluorescence detected at the tissue surface is determined by tissue morphology (both clinical and microscopic) and biochemistry. Cancer development in a number of tissues and organs has been characterized by a loss of normal autofluorescence. Such loss probably reflects multiple changes in the tissue (see discussion in ref. 3). The loss of autofluorescence in clinically occult high-risk oral lesions as shown in this study could reflect histomorphological changes (eg, dysplastic nuclei, thickening of the epithelium, and increased vascularization), and/or biochemical changes such as decreased density of collagen crosslinks (fluorophores), possibly owing to the breakdown of the extracellular matrix in response to the signals from the dysplastic cells and decreased flavin adenine dinucleotide concentration due to increased metabolic activity.

The promise of the optical technology has been shown by increasing numbers of studies, including those demonstrating alteration of autofluorescence in oral malignancies (reviewed in ref. 4). However, these studies have mostly
employed complex devices to measure spectroscopy indirectly using either photographic film or a sensitive or intensified charge-coupled device camera. Furthermore, these studies have focused on clinically visible lesion areas, and have not screened the rest of the oral cavity of cancer/dysplasia patients.

This is the first study to report noninvasive screening of not only the visible oral lesions but also the whole oral cavity of high-risk patients using an inexpensive, simple, hand-held robust device. The 3 case reports here are representative of our early findings in using this device for detection of occult lesions. This device is currently being evaluated within a longitudinal study in British Columbia that is following 200 patients with treated oral cancer and 200 patients with primary dysplasia for a total of 8 years. Approximately half of these patients have FVL oral lesions, with a third of these FVL lesions either completely or partially clinically occult under white light.

Patients with a history of oral cancer are known to have increased cancer risk. However, despite vigorous follow-up, many second primary tumors and recurrences are still not caught at an early preinvasive stage, partly due to their occult presentation. As illustrated in cases 2 and 3, fluorescence visualization could detect such change early during the management of such cases, allowing the disease to be identified before it can progress into an invasive cancer, and thus reducing mortality and morbidity. Remarkably, occult disease is also apparent in patients with primary dysplasia and is detectable using direct fluorescence visualization, as exemplified by case 1. Optical methods based on fluorescence imaging have previously been shown to have great promise in detection and localization of precancers in lung, cervix, skin, and oral cavity.11–19 This current report extends these studies, showing an additional role for the device in delineating occult disease present prior to invasion. As such, this technology may provide a mechanism by which the clinically nonapparent spread of abnormal cells might be monitored during studies of the natural history of the disease.

In summary, this early publication highlights a potentially significant contribution of fluorescence visualization to early detection of occult disease. Of interest, the device also appears to facilitate the visualization of high-grade occult disease at the margin of clinically apparent lesions, further supporting its use in detecting occult disease (unpublished data). The value of this device will have to be ascertained within longitudinal follow-up, but the data to date are encouraging.

Acknowledgments. We would like to acknowledge the assistance of Terrance Gilhuly of LED Medical Diagnostics in the construction of the direct visualization device.

REFERENCES


Appendix F
2007 ACR Annual Meeting  
April 14-18, 2007  
Los Angeles, CA

Abstract Number: LB-151  
Presentation Title: Direct fluorescence visualization as adjunct for identification of high-risk oral premalignancies  
Presentation Start/End Time: Tuesday, Apr 17, 2007, 8:00 AM -12:00 PM  
Location: Exhibit Hall, Los Angeles Convention Center  
Author Block: Lewei Zhang, Catherine F.Y. Poh, Samson Ng, Michele Williams, Denise Laronde, Calum MacAulay, Miriam P. Rosin. University of British Columbia, Vancouver, BC, Canada, BC Cancer Agency, Vancouver, BC, Canada

Purpose: To investigate the value of an easy, hand-held direct fluorescence visualization (FV) device, Velscope, in identifying high-risk oral lesions in Dysplasia Clinics in British Columbia, Canada. Method: 501 patients with a history of oral dysplasia or cancer were recruited from an ongoing Oral Cancer Prospective Longitudinal (OCPL) study and assessed with the VelScope for normal green autofluorescence (FV retention, FVR) or loss of autofluorescence (FV loss, FVL), excluding possible masking factors from recent surgery, trauma, or chronic mucosal inflammatory conditions. Results: 790 lesional fields were examined longitudinally with a total of 2731 examinations. Lesions were clinically apparent in 1727 of these exams: 48% were FVR and 52% FVL. Of interest, in 14% of exams, the FVL was present at former lesion sites that were no longer clinically apparent. In total, 419 biopsies were taken during follow-up: 119 with no dysplasia, 121 low-grade dysplasia, 122 high-grade dysplasia and 57 cancers. FVL was significantly correlated with the severity of histology, present in 24% nondysplastic, 73% low-grade, 94% high-grade lesions and 96% cancers (P < 0.0001). Of the 121 low-grade dysplasia, 14 progressed into high-grade dysplasias (11 FVL), and 7 progressed into cancers (6 FVL). Out of 40 FVL-persistent former lesion sites, 21 cases have developed the clinically visible lesions during follow-up. Among those biopsied (N=12), the result showed 6 high-grade, 3 low-grade and 3 non-dysplastic. The comparative biopsies were performed on the remaining 19 clinically non-apparent, FVL-persistent lesions, which revealed 6 cancers, 6 high-grade dysplasia, 5 low-grade dysplasia and 2 with no dysplasia. Conclusion: The data support the use of VelScope as an adjunct tool to identify high-risk oral lesions. (Supported by grants R01DE13124, R01DE17013, NIDCR, with salary support to CFP from CIHR)

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Appendix G
Evaluation of a Suspicious Oral Mucosal Lesion

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ABSTRACT

Dentists who encounter a change in the oral mucosa of a patient must decide whether the abnormality requires further investigation. In this paper, we describe a systematic approach to the assessment of oral mucosal conditions that are thought likely to be premalignant or an early cancer. These steps, which include a comprehensive history, step-by-step clinical examination (including use of adjunctive visual tools), diagnostic testing and formulation of diagnosis, are routinely used in clinics affiliated with the British Columbia Oral Cancer Prevention Program (BC OCPP) and are recommended for consideration by dentists for use in daily practice.

Approach

The diagnostic process begins with a history that includes a review of the patient’s chief complaint followed by completion of a thorough medical history. Once this has been obtained, a comprehensive clinical examination including extraoral, intraoral and mucosal lesion assessments should be completed. Only then can a diagnosis or a decision about the need for further investigation be rendered and appropriate decisions made regarding patient care.

History of the Current Illness

Over the course of a typical practice day, a dentist will examine the mouths of many patients. On occasion, a change in the oral mucosa will be detected. The challenge is to decide whether the abnormality requires further investigation. If the answer is yes, the British Columbia Oral Cancer Prevention Program (BC OCPP) team recommends a systematic approach to the evaluation of the lesion that includes methodical gathering of background information and a step-by-step clinical examination (Box 1). A methodical process is important given that many mucosal conditions have a similar appearance. A “quick look” provides insufficient information and may result in misdiagnosis and improper care. Although the recommended approach is appropriate for use in evaluating any mucosal condition, the focus of this article will be limited to one that can be used to evaluate the lesions that are more likely to be premalignant or an early cancer.
might have occurred — has the symptom improved, remained unchanged or worsened over time? Identifying significant aggravating or relieving variables may also be helpful. It is important to remember that most oral premalignant lesions or early cancers have few if any symptoms. Persistent oral sensitivity or a sense of mucosal “roughness” may be warning signs. If a lesion has persisted over time or if it has become larger or more symptomatic, it is of concern and warrants prompt and thorough investigation.

**Medical, Tobacco and Alcohol History**

A comprehensive medical history that includes attention to tobacco and alcohol use should be obtained at the time of all new patient examinations and updated at general dental recall. Remember that 75% of oral cancer patients are regular users of tobacco or alcohol, which are conventional risk factors. Information to be collected should include habit type, frequency and duration. More detailed information about these risk factors is included elsewhere in this special issue.1

Review of the medical history should include a list of current medications, as certain drugs may cause oral tissue changes with characteristics similar to premalignant or early cancer changes. (For a detailed list of medication-associated mucosal changes, see Neville and others.2) Notable examples of such drugs include immunosuppressive, anti-inflammatory and antihypertensive medications. Also, steroids delivered in inhaler, topical or oral form and other medications that dry the mouth increase risk of development of oral candidiasis, which often appears as whitish, nonadherent plaques.

Finally, information regarding previous cancer history (type and associated treatment) and any known dermatologic conditions should be gathered. Certain dermatologic conditions, such as lichen planus, can manifest cutaneously and as white lesions intraorally.

**Clinical Examination**

The clinical examination should always include extraoral and intraoral components.3 If a mucosal lesion is identified, a systematic approach to lesion assessment is recommended.

**Extraoral Examination**

Complete the extraoral examination first. This includes inspection of the head and neck region for asymmetry or swelling. Palpate the submental, submandibular, cervical and supraclavicular regions paying particular attention to size, number, tenderness and mobility of lymph nodes. A bimanual approach is recommended as it enhances the examiner’s ability to appreciate the characteristics of any mass and to make comparisons with the contralateral side. This is of particular importance in the neck where some lymph nodes lie under the muscles. In patients who have had a prior dental infection or surgical procedure in the head and neck region, it is common to find small, painless, freely mobile residual lymph nodes. However, if a lymph node is enlarged (i.e., > 1 cm in diameter) and palpably firm or fixed to adjacent structures, referral or further investigation is indicated. To complete the extraoral examination, inspect and palpate the lips and perioral tissues for abnormalities.

**Intraoral Examination**

Systematically inspect and palpate all oral soft tissues, as oral cancer can develop at any anatomical site. Particular attention should be given to high-risk sites, which include the lateral and ventral aspects of the tongue, floor of mouth and the soft palate complex.

**Lesion Inspection**

If a mucosal lesion is identified, additional attention to its characteristics is recommended. Oral premalignant lesions and early oral cancers are quite varied in appearance (Fig. 1); clinical characteristics can be used to help raise the level of suspicion that a lesion may be premalignant or an early cancer. However, remember that a biopsy of the lesion is required to establish a definitive diagnosis, as seemingly benign lesions may still pose a risk. Mucosal lesions can be predominantly white or red and have variable thickness and texture. A speckled red and white appearance, nonhealing ulceration or induration should signal a priority need for biopsy or referral.
Figure 2: Lesion characteristics to record when charting a lesion or ordering a biopsy.

Figure 2 summarizes the terminology and characteristics commonly used to describe lesions suspected of being premalignant or early cancer: location, size, colour, outline and texture. A leukoplakia is a white patch that cannot be rubbed off and cannot be characterized clinically or histologically as any other lesion. Homogeneous leukoplasias can be classified as homogeneous or nonhomogeneous. Nonhomogeneous leukoplasias are white lesions that are uniform in both colour and texture. They are predominantly white and have a smooth, thin or slightly wrinkled texture. Nonhomogeneous leukoplasias usually have a rough (leathery or granular) or speckled surface. If a nonhomogeneous leukoplakia contains a red component, it is called an erythroleukoplakia. In general, homogeneous leukoplasias are believed to carry a lower risk of transforming into cancer than nonhomogeneous leukoplasias. Erythroplasias, which are predominantly red lesions of the oral mucosa, carry the highest risk.

The outline or borders of the lesion should also be considered. Diffuse lesions, with irregular or ill-defined edges are more worrisome than discrete lesions. The presence of multiple lesions is considered more worrisome than a solitary lesion. As mentioned, the presence of a mucosal lesion at selected anatomic sites (lateral and ventral aspects of the tongue, floor of mouth and the soft palate complex) is of greater concern. Finally, leukoplakia size is also correlated with cancer risk, although the cutoff size for risk level remains speculative. Most oral lesions are < 2 cm and have a low cancer risk.

Figure 3 summarizes the key clinical features of high-risk and low-risk mucosal lesions. The details of a clinical lesion can be best captured in a high-resolution clinical photo. In BC OCPP-affiliated clinics, these images are obtained at each patient visit. Such documentation allows the dentist to note changes in the clinical appearance of the lesion over time, an important determinant of risk. Figure 4 shows changes in a premalignant lesion that progressed to cancer over time. Completion of a lesion-tracking sheet is a simple way to enter this information into the patient’s chart, where it is then readily accessible to all care providers.

Differential Diagnosis
Oral mucosal lesions can usually be simply grouped into 5 categories, known as the 5 Is: inherent (congenital or hereditary, e.g., white sponge nevus), inflammation (e.g., oral lichen planus, some variants of geographic tongue), infection (e.g., oral candidiasis), iatrogenic (e.g., drug-induced lichenoid reaction, frictional hyperkeratosis) and idiopathic (e.g., oral premalignant lesion or neoplasm). The first 4 categories must be ruled out before
classifying a lesion as a leukoplakia or an erythroplakia. An atlas of clinical lesions is a useful office reference.

**Adjunctive Visual Tools**

Adjunctive visual tools can enhance contrast between the lesion and the adjacent normal oral tissue. The BC OCPP team is currently using 2 approaches to lesion visualization: assessment of toluidine blue stain retention and, more recently, direct fluorescence visualization. The latter technique relies on tissue optics to assess mucosal lesions using a simple handheld device. In contrast to toluidine blue (which stains nucleophilic tissue components, primarily DNA), tissue fluorescence visualization detects a complex interplay of alteration to tissue structure and biochemistry that has been associated with premalignant disease and cancer at several sites. The BC OCPP clinical team routinely uses these approaches in tandem at its affiliated referral clinics. Use in community settings is being evaluated.

Although toluidine blue has an established validity in the detection of oral cancers, its value in identifying oral premalignant lesions is less well defined. In BC OCPP-affiliated clinics, virtually all oral premalignant lesions with high-grade dysplasia (severe dysplasia, carcinoma in situ) show positive retention of the stain. Of equal importance, data from an ongoing longitudinal study demonstrate a strong correlation between retention of the stain by leukoplakias and the presence of molecular clones associated with high cancer risk. Staining of an oral premalignant lesion is associated with a 6-fold elevation in risk of the lesion progressing to cancer.6

Tissue optics using direct fluorescence visualization reveals valuable additional information. Fluorescence visualization detects virtually all high-grade oral premalignant lesions and cancers and may play a critical role in the delineation of surgical margins and follow-up after treatment.7-9

**Figure 5** illustrates the potential value of combining these approaches to visualize oral lesions. Alone, these techniques are not diagnostic; however, in BC OCPP-affiliated clinics, they have been shown to enhance lesion characteristics, identify satellite or clinically non-apparent lesion sites and assist in biopsy site selection.
and timing of the biopsy. These techniques are complementary to and do not replace the comprehensive history and conventional visual and manual head, neck and oral examination. Good clinical judgment remains key in all circumstances.10,11

**Diagnostic Biopsy for Definitive Diagnosis**

Once the dentist has completed a thorough history and comprehensive clinical examination, he or she will need to decide which mucosal lesions can appropriately be monitored and which require biopsy. We do our patients a great disservice and burden the health care system unnecessarily if we order a biopsy on every mucosal abnormality seen.

During an oral cancer screening examination, if a suspicious mucosal lesion persists for more than 3 weeks following removal of local irritants, such as trauma, infection or inflammation, diagnostic biopsy(ies) or referral to an oral health care provider with expertise in the evaluation and management of premalignant or potentially malignant conditions is recommended. Tissue biopsy remains the gold standard for diagnosing an oral premalignant lesion or oral cancer. A carefully selected, performed and interpreted biopsy is critical in rendering an accurate diagnosis. Additional information on the biopsy procedure and interpretation of results is available in this special issue.12

Appropriate management decisions are made through the described approach to the evaluation of any mucosal lesion. A definitive diagnosis is an opinion based on critical analysis of all pertinent information obtained. Once the practitioner arrives at this conclusion, a decision about optimum patient care can be made.

**Conclusion**

In this paper, we describe a methodical approach to the assessment of oral mucosal conditions that are thought likely to be premalignant or an early cancer. This approach has been standardized throughout all BC OCPP-affiliated clinics. Members meet regularly to exchange ideas, update protocols, solve problems and discuss new program developments. Teamwork, including the integration of various disciplines and institutes, has been critical in the evolution of the oral cancer screening program. It ensures seamless patient management from the mildest premalignant change to frank malignancy. We hope that you will consider our resources and approach and adapt them for use in your practice. Together we can make a difference!
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The authors have no declared financial interests.

This article has been peer reviewed.

References

Appendix H
### Oral Abstracts – Tuesday, June 24

#### #1 - Time presenting: 14:30 – Venetian Room (Lobby Level)

| A REAL-TIME APPLICATION OF FLUORESCENCE VISUALIZATION (FV) TO IDENTIFY A NOVEL OPTICAL FIELD FOR SUBCLINICAL EXTENSION IN HIGH-RISK ORAL LESIONS. C. Poh, L. Zhang, S. Durham, D. Anderson, A. Kung, M. Rosin. University of BC, BC Cancer Agency & Research Centre, Vancouver. There is no consensus in managing high-grade dysplasia/carcinoma in situ (HGL). Frequent recurrence following excision implies the presence of subclinical change at the margins not-apparent at surgery, resulting in incomplete excision. FV has demonstrated the ability to identify clinically not-apparent oral lesions. The objective of this study is to apply this novel optical technology in the operating room to assess surgical fields for subclinical extension beyond clinical boundaries in high-risk oral lesions. Among 35 lesions (22 HGLs and 13 SCCs) examined, FV alteration (FV loss, FVL) was noted in all lesions. For HGL, almost all (21/22; 95%) FVL was going beyond clinical boundaries. This uneven subclinical extension of FVL, ranging from 1 to 25 mm did not differ significantly from those of SCC. Strikingly, 35% (13/37) of biopsies from FVL boundary beyond clinical boundary of HGLs showed histologically high-grade change and 5 of them beyond 10-mm, the conventional margin set-up for cancer. Conclusion: Through identifying subclinical field change associated with high-risk histology, integrating FV in surgery might provide a useful approach to better manage HGL at the point of care and a subgroup of HGLs should be treated aggressively (sponsored by NIDCR R01 DE17013, CIHR MOP-77663 and MSFHR). |
Sensitivity of direct tissue fluorescence visualization in screening for oral premalignant lesions in general practice

Kevin Huff, DDS, MAGD • Paul C. Stark, ScD • Lynn W. Solomon, DDS, MS

Various specialty clinics and research centers have conducted studies of direct tissue fluorescence visualization as a screening technique for oral premalignant lesions and early oral squamous cell carcinoma (OSCC). The effectiveness of the VELscope in a private practice setting is unknown. This pilot study is the first report to assess the VELscope system as a screening adjunct among lower-risk populations seen by a primary care clinician in a general practice setting. This study involved a retrospective comparison of two oral cancer screening examination protocols conducted on a presumably low-risk patient population seen in a private general dentistry practice. For one year, all patients age 12 or older received oral examinations, according to a standard oral cancer screening protocol. The following year, the same population was examined according to the same protocol with the addition of direct tissue fluorescence visualization using the VELscope.

Screening with incandescent light examination yielded a prevalence of mucosal abnormalities of 0.83%, none of which were premalignant. Screening with incandescent light examination combined with direct tissue fluorescence visualization yielded a 1.3% prevalence of mucosal abnormalities; based on surgical biopsy and histopathologic examination, 83% of these were potentially premalignant epithelial dysplasia.

Materials and methods

Over a two-year period, a general dentist performed oral cancer screening protocol. The following year, the same population was examined according to the same protocol with the addition of direct tissue fluorescence visualization using the VELscope.

Incorporating new screening technologies into private practice may be challenging. As new technologies are developed, general practitioners must decide whether to implement such adjunctive screening measures, basing their decisions on the current literature. This retrospective pilot study compared the efficacy of adding direct tissue fluorescence visualization to a standard oral cancer screening protocol performed in a private general dentistry practice.

A wide range of normal benign abnormalities—such as morsicatio buccarum (cheek biting), melanotic macules, amalgam tattoos, leukoedema, and so forth—may be confused as disease processes.

For many years, dentists screening for oral cancer have been limited to using incandescent light illumination to visually inspect the oral cavity and manual palpation. Recently introduced adjunctive screening technologies may allow clinicians to detect early epithelial dysplasia and OSCC. However, one cannot substitute any adjunctive screening device into the examination process as a replacement for the ability to recognize basic oral pathological findings and normal variations in the appearance of oral tissues. One sophisticated but easy to use screening modality is the VELscope (LED Dental, White Rock, British Columbia, Canada; 888.541.4614), a non-invasive, direct tissue fluorescence (narrow band) visualization technology. This device emits a particular wavelength and intensity of light that illuminates the oral mucosa and excited natural fluorophores in the tissue. The tissues emit fluorescence that is visualized through a filter by a human observer.

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Over a two-year period, a general dentist performed oral cancer screening protocol. The following year, the same population was examined according to the same protocol with the addition of direct tissue fluorescence visualization using the VELscope.

Screening with incandescent light examination yielded a prevalence of mucosal abnormalities of 0.83%, none of which were premalignant. Screening with incandescent light examination combined with direct tissue fluorescence visualization yielded a 1.3% prevalence of mucosal abnormalities; based on surgical biopsy and histopathologic examination, 83% of these were potentially premalignant epithelial dysplasia.
screening examinations on all recall patients age 12 and older. The same patient base was seen by the same practitioner, using one of two protocols for detecting clinically abnormal areas. All examined patients were ambulatory and were American Society of Anesthesiologists (ASA) Class III or less.

Children as young as 12 were included because of recent reports that OSCC occurs in younger populations without risk factors.7 Other recent reports have associated OSCC with risk factors that include human papilloma virus (HPV) infection, marijuana use, and periodontal disease, in addition to the long-recognized associations with tobacco and alcohol use and genetic predisposition.8-11 The assumption was made that all adolescent and adult patients are at risk for development of premalignant lesions.

Oral cancer screening examinations were performed during all periodic oral examinations (D0120) and comprehensive oral examinations (D0150 and D0180) for each patient age 12 or older.12 A documented clinical examination technique with incandescent light illumination, visual inspection, and manual palpation was used to perform a standard oral cancer screening examination.13

**Standard oral cancer screening examination**
From December 1, 2005 to November 30, 2006, 959 patients received a standard oral cancer screening examination using incandescent light illumination. Each examination was conducted as if the patient were a new patient who had not been examined previously by the same practitioner. However, patients who entered the practice as actual new patients during the second year of the study were excluded from the reported data so that a similar patient population could be compared. According to a computerized practice analysis for this 12-month time period, the overall dental practice consisted of 2,133 active patients between the ages of 12 and 99, with a mean age of 55. Of this patient base, 1,006 were male and 1,127 were female.

**Standard oral cancer screening examination and direct tissue fluorescence visualization**
From December 1, 2006 to November 30, 2007, 905 patients received a standard oral cancer screening examination using incandescent light illumination; in addition, visual screening was performed using a VELscope for direct tissue fluorescence visualization. Each examination was conducted as if the patient were a new patient who had not been examined previously by the same practitioner. However, patients who entered the practice as actual new patients during the second year of the study were excluded from the reported data so that a similar patient population could be compared. According to a computerized practice analysis for this 12-month time period, the overall dental practice consisted of 2,029 active patients between the ages of 12 and 100, with a mean age of 55. Of this patient base, 947 were male and 1,082 were female.

**Follow-up**
Areas of the oral cavity identified clinically and considered to be abnormal were documented for both groups and the patients were scheduled for a follow-up examination 14 days later, after any suspected etiologic agents were removed. Pathoneumonic normal variations of tissue, such as amalgam tattoos, varices, minor hemangiomas, morsicatio buccarum, and so forth, were not noted because they were considered to be non-pathologic. This protocol was established to minimize false positives produced by inflammation.

Identified lesions that persisted after 14 days were brushed using one of two sample collection methods: the Oral CDx brush test (CDx Laboratories, Inc., Suffern, NY; 877.672.5722) or liquid-based brush cytology (BD SurePath, BD Diagnostics, Burlington, NC; 800.426.2176). Samples collected by Oral CDx were submitted to CDx Laboratories, Inc. for analysis by a cytopathologist. Samples collected for liquid-based brush cytology were placed into SurePath solution and submitted to Tufts Oral Pathology Services in Boston for modified Papanicolaou staining and microscopic examination by an oral pathologist.

**Results**

**Standard oral cancer screening examination**
From December 1, 2005 to November 30, 2006, 8 of the 959 patients examined were found to have clinically abnormal areas of the mouth that persisted for 14 days or longer. Brush samples from these patients were collected and examined. Six of the samples had a result of no abnormality, while the other two samples were diagnosed as mild atypia, with further investigation warranted. The two atypical samples were referred for surgical biopsy; histologic diagnoses for both were benign. One case was diagnosed as pigmentation due to exogenous material; the other was reactive hyperkeratosis with normal cellular morphology.

**Standard oral cancer screening examination and direct tissue fluorescence visualization**
From December 1, 2006 to November 30, 2007, 12 of the 905 patients examined were found to have clinically abnormal areas of the mouth that persisted for
14 days or longer (Fig. 1 and 2). Brush samples from these patients were collected and examined. Analysis of the brushed specimens showed abnormal results in all cases; these specimens were referred for surgical biopsy. Two cases were histologically benign; one of these was diagnosed as lichenoid mucositis and the other as a squamous papilloma. The remaining 10 cases were diagnosed as epithelial dysplasia, a potentially premalignant change.

Assuming a constant rate of premalignant and malignant epithelial abnormalities from one year to the next in this stable patient population, the incandescent light examination yielded a 0.83% prevalence of mucosal abnormalities, none of which were premalignant. The incandescent light and direct tissue fluorescence examination yielded a 1.3% prevalence of mucosal abnormalities, 83% of which were potentially premalignant.

Discussion

The standard of practice requires dental practitioners to perform regular clinical screening examinations of the extraoral head and neck and intraoral soft tissues.\(^1\) The screening examination is meant to recognize gross tissue abnormalities and make a clinical provisional diagnosis and a decision on the appropriate management.\(^1\)

Screening for disease entails testing people who apparently are symptom-free from the disease in question, to differentiate between those who probably have the disease and those who probably do not.\(^3\) Usually, screening tools are highly sensitive but are not specific; in addition, they may have high rates of false positive results. A false positive result occurs when the clinical diagnosis of an abnormality is investigated by surgical biopsy but the tissue is histopathologically normal.\(^3\) A screening technique does not provide a diagnosis. A surgical biopsy with microscopic examination by a pathologist remains the standard for diagnosing oral mucosal disease.\(^1\)

The VELscope is a form of direct tissue fluorescence visualization that utilizes the loss of natural fluorescent characteristics of metabolic intermediaries to identify...
dysplastic and hypermetabolic activity.\textsuperscript{13,14} Inflammation may have a similar appearance to early dysplasia; however, a properly conducted diascopy may be useful for interpreting non-fluorescent findings at the time of the screening examination.\textsuperscript{17} Regardless of diascopic results, all irregular findings should be re-evaluated in 14 days and any persistent lesions should be investigated with biopsy, even if they respond to diascopy.

When screening identifies early or occult oral mucosal lesions, general dentists can use minimally invasive epithelial cell collection techniques as case finding and patient education tools. These tests may be used to decide whether a surgical scalpel biopsy is indicated. A recent review by Patton et al emphasized that brush cytology is not appropriate for sampling obvious long-standing developmental or submucosal lesions.\textsuperscript{18} The review also emphasized that the biopsy should be performed on patients with known oral dysplasia or OSCC confirmed by biopsy.\textsuperscript{4,22-24} Routine VELscope use has been challenged by the observation that the current literature pertaining to this particular device does not support all of the principles of evidence-based decision-making.\textsuperscript{5} Laronde et al emphasized the need to train dentists to use the device.\textsuperscript{6}

A recent review of adjunctive techniques for oral cancer examination called for additional study of the VELscope as an adjunct in low-risk populations and for primary care providers.\textsuperscript{6} The present study is the first to involve the VELscope as an adjunct for detecting occult abnormal mucosal findings in a low-risk general dental practice. The results of this retrospective, observational pilot study provide data for future studies in a community private practice setting.

**Conclusion**

In the present study, routine incorporation of the VELscope in the examination protocol for low-risk adolescents and adults in a general dental practice proved useful in identifying occult, potentially premalignant lesions.

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**References**


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Appendix J
A new paradigm in the way we envision tissue change during carcinogenesis has evolved in recent years. From a clinical standpoint, this paradigm has altered how we view “at-risk” tissue. Rather than focusing on clinical lesions, we often discuss “field” changes involving the expansion of genetically and epigenetically altered cells within a tissue, and not necessarily centered on a clinically identifiable lesion. This change reflects the recognition that genetically altered fields of cells are not always clinically or histologically apparent, yet even when occult, can constitute a significant risk. This shift in perspective has caused a management conundrum. We can use molecular techniques to characterize field changes in an extremely detailed fashion; however, such evaluation depends on identifying areas for its use.

In this issue of the journal, Roblyer et al. (1) describe work with autofluorescence imaging, a field-assessment approach that may be an alternative and potential complement to lesion-focused assessments and may improve our ability to clinically distinguish normal from premalignant and malignant oral tissue in a real-time fashion. Generally, autofluorescence imaging uses higher-energy light to excite specific compounds in tissue (fluorophores) so that they re-emit lower-energy light that makes up the autofluorescence image of the tissue. The excitation light is produced by a filtered arch lamp, an array of light-emitting diodes, or a laser. Effective detection of the autofluorescence with digital imaging and digital processing of the images so as to move from a qualitative to an objective quantitative assessment. Although results are still preliminary, the data are interesting and suggest one way in which this approach could be extended to other clinical niches.

Changes in fluorescence reflect a complex interplay of alterations to fluorophores in tissue and structural changes in tissue morphology. The endogenous fluorophores most relevant to optical screening and diagnosis of precancer and cancer are those that excite in the spectrum from visible violet/blue (400-450 nm) to UV-A (315-400 nm) and have properties that have been spectroscopically correlated with disease progression (10–13). When tissue is illuminated in such a fashion, most of the fluorescence originates from collagen, its cross-links and elastin, which are located in the stroma and basement membrane, and a small fraction originates from the reduced form of NADH and the oxidized form of flavin adenine dinucleotide in epithelial cells. The interaction between the light source and tissue is also affected by characteristic changes of cancer development, such as alterations to epithelial thickness, nuclear morphology (dysplastic nuclei), and vascularization, that affect absorption and scattering of light.

What has autofluorescence told us to date about at-risk oral fields? Our group has been using direct FV to follow patients enrolled in the Oral Cancer Prediction Longitudinal Study in Vancouver, British Columbia. An intriguing early discovery was that this technology identified lesions not apparent under white-light examination yet containing dysplasia and/or cancer when biopsied (14). Equally remarkable, even lesions apparent under conventional white light frequently involved autofluorescence loss extending into the surrounding tissue with no apparent clinical change. In 2006, we showed for the first time that direct FV could be used with the commercial release of the VELscope® (LED Dental, Inc., White Rock, British Columbia, Canada), which is approved by the U.S. Food and Drug Administration and Health Canada. This simple handheld device uses a blue/violet light (400-460 nm) to illuminate oral tissue. A selective filter in the eyepiece allows the viewer to directly visualize the pale green autofluorescence that is given off by normal tissue (9). Abnormal or suspicious tissue shows decreased levels of normal autofluorescence and appears as a dark brown to black region in comparison with the brighter, green surrounding healthy tissue.

Although early results with autofluorescence in the oral cavity have been promising, published data to date have come mainly from oral specialists working in referral clinics. Roblyer and coworkers describe a potentially important step in the evolution of this approach toward facilitating its transfer to less-experienced clinicians, including those in community settings. Departing from the direct visual assessment of autofluorescence such as with a VELscope®, they coupled autofluorescence with digital imaging and digital processing of the images so as to move from a qualitative to an objective quantitative assessment. Although results are still preliminary, the data are interesting and suggest one way in which this approach could be extended to other clinical niches.

Perspective

Tracing the “At-Risk” Oral Mucosa Field with Autofluorescence: Steps Toward Clinical Impact

Perspective on Roblyer et al., p. 423

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used in the operating room to identify subclinical high-risk fields (15). This study examined 20 consecutive patients undergoing surgical excision, documenting histologic and molecular changes within areas showing loss of autofluorescence in clinically normal tumor margins. All tumors showed autofluorescence loss, which extended beyond the clinically visible tumor boundary in 19 cases. Of note, autofluorescence loss was not uniformly distributed around the clinically apparent lesion, varying in extent and evident from 4 to 25 mm beyond the clinical parameter. It is striking that autofluorescence loss in these clinically occult margins was associated with both histologic change and the presence of high-risk molecular clones even in the majority of margins with low-grade or no dysplasia.

Will the use of FV to guide surgery reduce tumor recurrence? The literature documents a high frequency of recurrence at the primary site (10-30% of cases) of oral cancer (16–18). We have begun a longitudinal study to explore the effect of FV in defining the surgical margin on outcome of oral cancer surgery. Between 2004 and 2008, 60 patients with a ≤4 cm oral cancer entered the study. Each case was treated with surgical excision alone and was followed for at least 12 months. Thirty-eight patients had FV-guided surgery, with the surgical margin placed at 10 mm beyond the perimeter of autofluorescence loss. The remaining patients (control group) had the surgical margin placed at 10 mm beyond the tumor edge defined by standard white-light examination. Use of the FV-guided approach depended on the availability of the pathologist to attend the surgical procedure.

The FV-guided and control groups had similar distributions with respect to gender, smoking habits, anatomic lesion site, and follow-up time. To date, 7 of the 60 cases (12%) have developed a recurrence of severe dysplasia or worse neoplasia at the treated site, all in the control group (25% versus 0%, P = 0.002). Analyses of histologic and molecular alterations in tumor margins are ongoing (Fig. 1). These data suggest the utility of autofluorescence changes within this clinical setting and provide pilot support for planning a larger clinical trial aimed at establishing whether FV-guided surgery has value.

We are also using FV to monitor the potential re-emergence of regions of autofluorescence loss at treated sites in the cases accrued to the longitudinal study and are currently completing an interim assessment of these monitoring results. Autofluorescence loss persists in some cases, increasing in size and intensity over time and giving rise to a clinical lesion containing dysplasia or cancer (examples in Fig. 2 and ref. 19). New lesions showing autofluorescence loss at some distance from the treated site have also been observed. Molecular analysis is being used to determine whether this is recurrent disease or a second primary (18, 20–22).

What do the autofluorescence modifications of Roblyer et al. contribute to this field? Their study represents the first use of multiple fluorescence excitation and reflectance imaging to detect and delineate oral cancer and premalignant lesions. It reflects a growing trend toward multimodal and quantitative imaging for early cancer detection using color ratio imaging (6, 23, 24). The authors used this approach to select an optimum wavelength that may more precisely
By excluding confounders in an objective fashion would be an important step towards making autofluorescence available to inexperienced users in community settings, in which potential confounders are more frequent than are oral malignancy and premalignancy.

Roblyer et al. used a wide-field multispectral digital microscope, which essentially is a modified dissecting microscope, to illuminate tissue with 365, 380, 405, and 450 nm excitation. The resultant autofluorescence images were recorded with a high-resolution charge-coupled device color camera (red, green, and blue channels). Sixty-seven subjects (56 patients and 11 normal volunteers) were evaluated with 276 measurements from 159 regions of interest. This data set was divided into a training and a test set; the training set was used to select the best illumination wavelength and color combination for discriminating normal from abnormal areas. A red channel-to-green channel ratio for 405 nm excitation provided the best discrimination between neoplastic (including dysplasia, carcinoma in situ, and cancer) and nonneoplastic areas (including inflammation and hyperplasia) in the training set, and its performance was validated in the test set. The classification algorithm developed in this way had 96% sensitivity and 96% specificity in the training set and 100% sensitivity and 91% specificity in the test set.

In a further effort to make the technology more accessible to primary care providers, the authors have produced a probability map that overlaps and highlights abnormal areas within a white-light image of the oral cavity. This probability map is based on the red-to-green ratio in the classifier and is used to indicate areas of high-likelihood of abnormality versus low-likelihood of abnormality. Disease-probability maps were compared with histologic diagnosis of tissue resected from the field of view. The authors noted that there was a qualitative agreement between the presence of dysplasia and cancer as indicated by the map and the corresponding histology.

Will the modifications proposed by Roblyer and coworkers facilitate the uptake, further evaluation, and quality of data obtained with autofluorescence in the community setting, as suggested by the authors? It is too soon to judge. However, these are important first steps in the evolution of this approach toward an improved ability to address clinical needs.

As mentioned by Roblyer et al., their present data represent a strong proof-of-principle for the approach. However, the range of lesions being evaluated in any clinical setting is notoriously heterogeneous, and only a fraction of these abnormalities were available to the researchers in the tertiary care setting. One of the most critical future steps will be the expansion of this analysis to a larger number of cases, carefully choosing a spectrum of lesions that reflects the wide range of clinical abnormalities encountered for each clinical use that is being targeted, for example, in screening versus diagnostic settings.

For screening, this spectrum should include changes that are common to the general population and that are known to confound autofluorescence assessment, such as inflammation (e.g., lichen planus), infection, and chronic trauma. The current assessment was limited to only a few such examples.

For diagnostic settings, the further characterization of dysplastic lesions and hyperplastic lesions detected with FV loss will be equally crucial. Our experience within the ongoing longitudinal study has been that nearly all high-grade dysplasias (severe dysplasia/carcinoma in situ) show FV loss. This finding needs to be confirmed more broadly in a multicenter study. In contrast, only a portion of low-grade (mild or moderate) dysplasia shows FV loss. Autofluorescence determinations need to be combined with molecular analyses to determine whether high-risk molecular clones are more prevalent in low-grade lesions showing autofluorescence loss (versus not; Fig. 1). These low-grade lesions also need to be followed over time to determine the likelihood of outcome for different combinations of molecular and autofluorescence changes. Last, the key to our understanding of autofluorescence-detected fields is the collection of concise and detailed descriptions of both lesions (including pictures) and test subjects (demographics, risk factors, and medical history). These data will allow for a later pooling of results within a meta-analysis framework. Critical information for premalignant disease includes the degree of dysplasia and whether the dysplasia occurs in patients with former oral cancers, as part of a cancer, or as a primary dysplasia (i.e., no history of oral cancer.

Fig. 2. A 63-year-old female former smoker was examined for 6 mo (A and B) and 12 mo (C) after a surgical excision of severe epithelial dysplasia on the left lateral tongue. A, white-light image of a well-healed scar on the left lateral tongue (arrow); B, the anterior aspect of this scar (arrow) under fluorescent visualization showing a dark brown region of autofluorescence loss; C, at 12 mo, the same area (arrow) showed a persistent autofluorescence loss of increased size; at 20 mo after initial treatment (data not shown), a biopsy from the region of loss (arrow) showed carcinoma in situ.
or dysplasia). Risk of progression is known to change with each of these aspects.

The Roblyer et al. development of probability maps is also intriguing. Further advances in this process will most likely involve a combination of technology change and the continuing integration of the data described in the previous paragraph. For example, although the autofluorescence images in the article are promising (1), they will need to become more robust to the effects of noise (random fluctuations of the data in the image), particularly in the darker areas, where small changes in the denominator (green channel) could result in large fluctuations of the ratio used to calculate the disease-probability values. To go to real-time probability mapping, software improvements will be needed for determining the red-to-green ratios and for displaying the color code (the range of map colors indicating low-to-high probability of neoplasia) as the images are being acquired. Nevertheless, this approach has promise for the quantitative establishment of the presence and boundaries of a lesion.

In summary, autofluorescence and other such imaging processes have the potential to make a significant impact on standards of care, influencing not only surgical margin assessment but also the evaluation of tissue alterations during chemoprevention, which relies on establishing lesion boundaries. Autofluorescence imaging has already begun to shed new light on tissue changes during cancer development (25). Other visualization technologies (e.g., in vivo confocal microscopy, molecular targeted optical contrast agents alone and in combination) are poised to enter this field and will be integrated in the future with molecular findings to further advance our understanding of disease processes. How this is to be done will be complex; for example, the field defined by existing optical agents such as toluidine blue (26) does not overlap completely with that defined by direct FV, and neither completely corresponds with a molecularly altered field. Only long-term follow-up of patients can unravel the clinical significance of these different views of the underlying neoplastic process.

A major challenge for any new technology, including that presented by Roblyer et al., is to develop a mechanism by which we can begin to collect these very diverse sets of information into a framework that will facilitate its use to address important clinical questions.

**Disclosure of Potential Conflicts of Interest**

Dr. Calum MacAulay serves on the scientific advisory board of Remicalm LLC.
Appendix K
Assessment of the VELscope as an Adjunctive Examination Tool

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The VELscope is a visualization-enhancement adjunct currently marketed to assist the clinician in assessing potentially dysplastic or malignant mucosal lesions in the oral cavity. The author examined 130 subjects, who smoked at least one package of cigarettes per day. The clinical findings derived from a conventional examination protocol were compared to the findings observed with the VELscope. Ten suspicious lesions were identified by conventional examination. No occult suspicious lesions were identified using the VELscope. VELscope interpretation did not enhance or otherwise alter the clinical management of the suspicious lesions. Several commonly occurring conditions, such as mucosal pigmentations, ulcerations, irritations, and gingivitis were associated with a loss of fluorescence using VELscope. The findings of this study raise questions concerning the utilization of the VELscope as a screening adjunct.
Oral health care providers play a pivotal role in the early detection of pre-malignant and malignant conditions affecting the oral and pharyngeal complex (1-3). However, there is some concern that clinicians are not providing patients with a thorough soft tissue examination on a routine basis (3-5). The presumed consequence is that the majority of intraoral oral cancers are diagnosed at a later stage and the overall 5 year survival rate has remained relatively constant at about 55-59 percent over the past several years (3, 6). One response to this situation is the introduction of new adjunctive technologies promoted as improving the practitioner’s ability to identify oral premalignant and malignant lesions (OPMLs) (7). The VELscope (LED Dental, White Rock, British Columbia, Canada), was recently introduced as a visualization adjunct (Figure 1). The utility of the VELscope is based on the premise that dysplastic and malignant tissues manifest different spectral characteristics (fluorescence) compared to surrounding healthy tissues.

Figure 1. VELscope oral cavity fluorescence detecting device.

Figure 2. Ulceration soft palate.

Figure 3. VELscope image ulceration soft palate.

The concept of assessing the fluorescent characteristics of mucosal tissues to identify areas of potential dysplasia or malignancy has been a topic of ongoing research for over 40 years (4, 8-20). Initial research focused predominately on increased levels of porphyrins as the signature marker for dysplasia or malignancy (8, 14, 15, 17). Porphyrins are intermediate products created during the synthesis of metaloporphyrins such as heme. Unfortunately, bacteria-produced porphyrins are commonly observed in the dental plaque and dorsal tongue, prompting questions as to the reliability of porphyrins as a marker of concern (8, 11). Contemporary research has focused on the change in the levels of endogenous fluorophores such as nicotinamide adenine dinucleo-
The premise for VELscope’s utility is based on fluorescent changes demonstrated in dysplastic and malignant tissues caused by decreased collagen-cross links, increased hemoglobin absorption and to a lesser degree increased scattering in the epithelium, epithelial thickening, and reduced FAD concentrations (23). VELscope has been cleared by the Food and Drug Administration (FDA) for the direct visualization of oral-cavity fluorescence (25, 26). However, there is no published information addressing the utility of the VELscope as an adjunct to routine examination adjunct (7, 27-30).

The objective of this pilot study was to assess the value of the VELscope as an adjunctive tool in the examination of 130 subjects who smoked at least one package of cigarettes per day.

Methods

A convenience sample of 130 individuals were included in the study. Inclusion criteria included: males and females over 18 years of age who smoked at least one pack of cigarettes per day but were otherwise healthy. As there was a single examiner, no calibration was deemed necessary. Study protocol approval was obtained from the Institutional Review Board, The University of Texas Health Science Center San Antonio, San Antonio, Texas. Participants signed an informed written consent and completed a standard personal and medical history, which was reviewed by the examiner.

A conventional oral soft tissue examination was performed by visual inspection using incandescent overhead and halogen dental illumination accompanied by digital palpation. Abnormal epithelial changes associated with the gingiva; labial, buccal, and vestibular mucosa; hard and soft palate; oropharynx; edentulous alveolar ridge; dorsal, lateral and ventral surfaces of the tongue; and floor of the mouth were recorded. Immediately thereafter, direct visualization of oral-cavity fluorescence was accomplished utilizing the VELscope in accordance to the manufacturer’s guidelines. All the aforementioned oral tissues were assessed and documented. Subjects with suspicious lesions were scheduled for a follow-up examination and a biopsy where indicated. The results of histological evaluation were discussed with the subjects and appropriate periodic reevaluations or referrals were arranged.
Results

Between August 2007 and May 2008, a total of 72 males and 58 females were recruited in the study. The age range was 19 to 79 years of age (average = 46.) The self-estimated pack years of smoking ranged from 1 to 70 (average = 27). Conventional examination identified 10 subjects with suspicious clinical lesions requiring monitoring or biopsy. No additional lesions were identified by VELscope interpretation. The management of the ten suspicious lesions was not altered by VELscope interpretation.

The findings of the conventional oral examination when compared with the findings obtained using the VELscope were dichotomous. The absence of a distinct clinical lesion visualized by conventional examination or the visualization of pale green fluorescence using the VELscope was defined as within normal limits (WNL). Clinical lesions noted on conventional examination were described and documented, while the visualization of a distinct dark green to black loss of fluorescence (LOF) using the VELscope was documented as an “LOF” result.

The corresponding VELscope results for the numerous benign conditions identified by conventional examination were variable. Seventy two percent (80/111) of lesions or conditions clinically characterized by inflammation or pigmentation demonstrated LOF. In contrast, 98 percent of conditions characterized by some form of keratotic thickening (e.g. linea alba, frictional keratosis, leukoedema) were within normal limits (Figures 2, 3, 4, 5).

Discussion

This is the first study to report on the utilization of the VELscope in the assessment of some of the common mucosal lesions observed in general practice. While the subject population was restricted to smokers, it otherwise represented the profile of patients likely to be seen in the general practice setting. The VELscope examination did provide an additional perspective for the investigator to assess mucosal tissues. However, this additional information did not alter clinical management.
Assessment of the VELscope

Previous investigations of the VELscope have focused on LOF in suspected dysplasia or squamous cell carcinoma (SCC). In a study of 44 patients with biopsy-confirmed dysplasia or SCC, Lane and colleagues utilized the prototype VELscope to perform direct fluorescence visualization of 50 mucosal lesions that were subsequently biopsied (23). The decision to biopsy was based on traditional clinical features (patient history, clinical appearance, toluidine blue staining). Comparing the fluorescent findings with the histopathological diagnoses, the authors calculated a sensitivity and specificity of 98 percent and 100 percent, respectively. Poh and colleagues reported on the use of the prototype VELscope in a case study of three patients with previously diagnosed dysplasia or carcinoma in situ (31). In one case, an area of decreased fluorescence that was not associated with a visible area of suspicion was biopsied and diagnosed as moderate epithelial dysplasia. In two other patients, a loss of fluorescence prompted the histologic assessment of two areas of erythema that were initially deemed innocuous by routine visual examination. Both of these lesions were diagnosed as carcinoma in situ on histological examination. These studies highlight the use of the VELscope to validate clinical impressions and define tumor margins in established malignant or premalignant lesions (32).

While these studies support proof of concept, it was not the intention of the studies to evaluate the VELscope’s usefulness in routine dental examinations. The study cohorts included patients previously diagnosed with dysplasia or carcinoma who were being followed in a specialty clinic. Such referral-based populations do not represent the population typically seen in a general dental practice. There was no assessment of the fluorescent characteristics of the multitude of other mucosal lesions commonly observed in clinical practice.

Betz and colleagues noted vast differences in fluorescent intensities for different oral cavity locations (8). In an attempt to better define the fluorescent character of normal oral mucosa sites, de Veld and colleagues studied 97 healthy men and women without regard to racial distinction and concluded spectral similarity among all oral mucosal tissue sites,
except for dorsal tongue and the vermilion border of the lip (11). However, in a follow-up study assessing 96 subjects with no clinical oral lesions, de Veld and colleagues reported that individual fluorescent characteristics were significantly affected by skin color and to a lesser degree by gender, tobacco use, and alcohol consumption (12). As a consequence, the authors question the utility of using fluorescence spectroscopy to evaluate patients with strong oral pigmentation.

Commonly encountered inflammatory conditions, such as cheek biting, gingivitis, and viral eruptions frequently demonstrated LOF on VELscope examination. Areas rich in lymphoid tissue also appeared to alter VELscope interpretation. While one subject in the current study manifested a worrisome asymmetrical tissue proliferation on the left posteriolateral aspect of the tongue with associated LOF, there were three comparable instances associated with LOF associated with a sore throat.

In regard to specific lesion characteristics in the current study, flat epithelial lesions appeared to be more amenable to fluorescence interpretation than exophytic lesions (8). It has been postulated that mucosal thickening adversely attenuates fluorescence emanating from the underlying submucosa. This may explain the unremarkable VELscope finding associated with a malignant verrucoid mass in the left cheek. However, such limitations pertaining to lesion depth may dampen the value of the VELscope in assessing some lesions.

In the present study, a clinical reassessment of the many aforementioned incidences of LOF was all that was necessary to allay any examiner or patient concern. However, the sheer preponderance of such occurrences and the multitude of variables associated with each have led some to question the utility of using fluorescent spectroscopy to distinguish OPMLs from benign lesions in the oral cavity (13).

These observations and concerns regarding the use of the VELscope should not be interpreted as a definitive dismissal of its potential value as an examination adjunct. As this was an observational pilot study, limitations of the study must be acknowledged. First, there was no examiner blinding and while the investigator felt confident in his ability to consistently interpret both the clinical and the VELscope findings, the potential for both bias and misinterpretation must be considered. Secondly and perhaps more importantly, the lesions deemed by the investigator to be non-suspicious were not subjected to histologic confirmation. While this approach represents the real-world manner in which clinicians frequently assess oral lesions, it does not allow for a determination of sensitivity and specificity.

Ultimately, the findings of this study raise several questions concerning the utilization of the VELscope as a general screening adjunct. The VELscope does afford the examiner additional information for interpretation. For practitioners well-versed in its use, the VELscope may be a useful adjunct, especially in the monitoring of established lesions. However, in the present study, no suspicious occult lesions were uncovered and the management of the 10 suspicious lesions noted on conventional examination was not altered due to VELscope interpretation.

Finally, the previously noted concern that clinicians may not be performing thorough soft tissue examinations on a routine basis must be addressed (3-5). In order to improve the dental profession’s success in identifying OPMLs, the primary focus of the VELscope use should be to ensure that clinicians perform their profession obligation: to do a thorough soft tissue examination on a regular basis. In this regard, the developers of the VELscope are to be commended for their efforts to both raise awareness and educate the practitioner of this need.

**Summary**

In a comparative observational study of 130 subjects who smoked at least one package of cigarettes per day, 10 suspicious oral lesions requiring follow-up monitoring or biopsy were discovered through routine clinical examination. No occult suspicious lesions were discovered using the VELscope. VELscope interpretation did not enhance or otherwise alter the clinical management of the suspicious lesions. Several commonly occurring conditions such as mucosal pigmements, ulcerations, irritations, and gingivitis were associated with a loss of fluorescence. More study is indicated to determine the true value of routine use of the VELscope in routine dental examinations.
Acknowledgements

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A Cross-Sectional Study Evaluating Chemiluminescence and Autofluorescence in the Detection of Clinically Innocuous Precancerous and Cancerous Oral Lesions
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A cross-sectional study evaluating chemiluminescence and autofluorescence in the detection of clinically innocuous precancerous and cancerous oral lesions

Ravi Mehrotra, MD; Mamta Singh, MD; Shaji Thomas, MDS; Preeti Nair, MDS; Shruti Pandya, MSc; Niraj Shakti Nigam, BDS; Pankaj Shukla, MD

Cancer of the oral cavity is the sixth most common malignancy reported worldwide, and it has one of the highest mortality rates among all cancers.1 In 2008, an estimated 35,000 people developed cancer of the oral cavity and oropharynx in the United States, and approximately 7,500 people died of the disease.2 In India, oral cancer is the most prevalent cancer in men and the third most prevalent cancer in women, and it makes up 40 percent of all cancers in the country.3 Early diagnosis of oral cancer greatly increases the probability of achieving a cure with minimum impairment and deformity.

Light-based oral cancer screening aids have been developed with the stated goal of assisting dentists in

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ABSTRACT

Background. ViziLite Plus with TBlue system (Zila Pharmaceuticals; now Zila, a division of Tolmar, Fort Collins, Colo.) and VELscope (LED Dental, White Rock, British Columbia, Canada) are oral cancer screening aids that have been developed to assist dentists in identifying precancerous and cancerous oral lesions.

Methods. The authors screened patients with an overhead examination light and then with VELscope or ViziLite. Patients with a clinically innocuous lesion underwent a biopsy, and the authors compared the results of tissue pathological analysis with findings from the screening aid tests to determine the sensitivity and specificity of each device. The authors tested these devices to determine their ability to aid in the decision-making process regarding whether further evaluation of a clinically innocuous lesion was required.

Results. The authors biopsied 102 lesions and examined them with the ViziLite. They found three dysplasias and one malignancy, none of which were detected with the ViziLite (sensitivity = 0 percent, confidence interval [CI] = 0-60.2 percent; specificity = 75.5 percent, CI = 66.7-82.8 percent). The authors biopsied another 156 lesions and examined them with VELscope. They found 11 dysplasias and one malignancy, six of which were detected with VELscope (sensitivity = 50 percent, CI = 21.1-78.9 percent; specificity = 38.9 percent, CI = 30.8-46.9 percent).

Conclusions. The study results indicate that use of ViziLite or VELscope along with a conventional screening examination for lesions deemed clinically innocuous was not beneficial in identifying dysplasia or cancer. Additional clinical studies are needed before these devices can be recommended.

Clinical Implications. Clinicians and patients could have a false sense of security after obtaining a negative ViziLite or VELscope examination result because potentially large numbers of precancerous and cancerous lesions will be missed by both devices.

Key Words. Oral cancer; dysplasia; oral cancer screening aids.

JADA 2010;141(2):151-156.
identifying precancerous and cancerous oral lesions at their earliest stage. Specifically, these devices are intended to be used as adjuncts to the conventional oral cavity examination to help visualize potentially dysplastic and cancerous oral lesions. We evaluated two of these products in this study: the ViziLite Plus with TBlue system (Zila Pharmaceuticals; now Zila, a division of Tolmar, Fort Collins, Colo.) and VELscope (LED Dental, White Rock, British Columbia, Canada).

Investigators assess the accuracy of an oral cancer screening aid by comparing the screening aid findings with those of pathological testing in a masked fashion (that is, the clinician using the screening aid is unaware of the patient’s pathological diagnosis and the pathologist is unaware of the findings from use of the screening aid) and in a general population setting. To date, no published prospective clinical trials, to our knowledge, have evaluated the ability of ViziLite or VELscope to detect oral precancerous and cancerous lesions when used as a screening tool. Consequently, recent reviews in the literature of these devices have questioned the benefits of these light-based systems because their accuracy remains unknown.

Unlike new pharmaceuticals and medical devices that require approval by the U.S. Food and Drug Administration (FDA) before they can be marketed in the United States, certain grandfathered medical devices such as ViziLite and VELscope may be marketed without FDA approval. If a manufacturer claims that a medical device is “substantially equivalent” to another medical device that was sold before 1976 (when the FDA first began regulating medical devices), the FDA may grant a 510(k) clearance that allows the manufacturer to market that device without substantial review of its safety and efficacy.

The 510(k) clearance of the ViziLite Plus with TBlue system was based on the manufacturer’s claim that the device was “substantially equivalent” to colposcopy examination lights sold to illuminate the uterine cervix during a gynecologic examination. The 510(k) clearance of VELscope was based on the manufacturer’s claim that it, in turn, was “substantially equivalent” to the ViziLite system.

The purpose of our study was to evaluate the use of these two systems as adjunct aids in diagnosing lesions deemed clinically innocuous according to conventional light examination. We also assessed the sensitivity and specificity of ViziLite and VELscope in the identification of oral dysplasia and carcinoma by independently comparing pathological examination results with those obtained with these visual screening aids.

**PARTICIPANTS, MATERIALS AND METHODS**

In June 2008, 258 patients seeking dental care and found to have clinically innocuous lesions were investigated across a 10-day period by a team of dental and medical specialists (R.M., S.T., P.N.) in the outpatient department of the government-run District Hospital in the Vidisha district in the state of Madhya Pradesh in central India. The team included specialists in oral medicine (P.N.), oral and maxillofacial surgery (S.T.) and oral pathology (R.M.). All three specialists had received significant clinical training and had considerable experience with both the ViziLite and VELscope devices to ensure reproducible and accurate clinical findings and screening aid results. However, we did not calibrate the examiners. The institutional ethical committee of the District Hospital at Vidisha approved the study.

We enrolled in the study patients who were 18 years and older after they provided written consent. One of the three specialists examined each patient with a conventional overhead light; we then assigned patients randomly to either the VELscope or ViziLite devices depending on which examiner screened them. The specialists rotated between the two devices to prevent fatigue as well as to ensure unbiased selection of patients. Before the examination, patients rinsed their mouths thoroughly with water.

We defined all identified oral lesions according to Sciubba’s definitions:

- **Class I**: lesion “causing suspicion of intraepithelial neoplasia” or frank malignancy necessitating immediate biopsy;
- **Class II**: clinically innocuous lesion “that in the investigators’ opinion required no further attention other than clinical follow-up.”

**Exclusion criteria.** We excluded patients with Class I lesions detected with a conventional overhead examination light (and referred them for treatment) and those without any oral lesions. We included patients with Class II lesions for subsequent evaluation with the light-based adjunct screening tools. Furthermore, we excluded oral lesions that were submucosal (for

**ABBREVIATION KEY.** FDA: Food and Drug Administration.
example, cyst, salivary gland tumor) or covered with a clinically intact normal epithelium (for example, hemangioma, fibroma). In addition, we excluded from the study patients with pigmented lesions such as nevi and amalgam tattoos and lip lesions, specifically those on the vermillion border or cutaneous surfaces, as well as patients who refused to undergo a scalpel biopsy. Finally, we excluded patients with medical problems and those who wore dental appliances, such as orthodontic or other fixed prostheses, that might interfere with the examination.

**Intraoral examinations.** The clinicians performed the examinations with the VELscope and ViziLite devices according to the manufacturers’ instructions. In addition to evaluating the Class II lesion, the clinician examined the entire oral cavity of every patient with the light-based screening aid in an attempt to identify new lesions not apparent during the oral examination with the conventional overhead light. It would have been best for all patients to be examined by multiple examiners with both VELscope and ViziLite. Unfortunately, we conducted this screening at a rural facility with time constraints and limited resources. Only one VELscope device and a limited supply of ViziLite kits were available.

Patients underwent an examination with the conventional overhead light and then, depending on which screening aid was available, underwent an examination with VELscope or ViziLite. The assignments were completely random. As explained earlier, we excluded patients with Class I lesions (that is, suspicious enough to warrant a biopsy). Consequently, they did not undergo examinations with the light-based devices because the examiners already had determined that they needed to undergo a biopsy. Findings with the light-based devices for these patients—whether positive or negative—would be meaningless.

After a participant rinsed with a dilute 1 percent acetic acid solution and the clinician examined the mouth with a chemiluminescent light, normal mucosa—a negative ViziLite finding—appeared blue or dark, while abnormal mucosa—a positive ViziLite finding—appeared acetowhite. The ViziLite Plus with TBlue system also contains a toluidine blue dye, which is intended to be used only to mark lesions for follow-up examination that are positive according to the ViziLite screening. The manufacturer claims no diagnostic capability for the dye.

The VELscope is a portable device that is used to examine the oral cavity. Normal mucosa—a negative VELscope finding—appears as a bright green glow, while abnormal mucosa—a positive VELscope finding—is identified by a loss of fluorescence and appears dark.

Three of us (R.M., N.S.N., P.S.) obtained demographic information about each patient, including age, sex and tobacco use. The examiners performed detailed clinical examinations in each patient to assess the site and size of all oral mucosal lesions, and they recorded this information on a standard form.

Using the standard scalpel technique, two of us (S.T., P.N.) obtained biopsy samples from patients who were under local anesthesia. The samples were processed at laboratories in Mumbai, India. Hospital pathologists first analyzed the specimens and then an independent pathologist with expertise in oral dysplasia and cancer analyzed the specimens; we used the independent pathologist’s findings in the final data analysis. The pathologists were masked with regard to the clinical data and screening aid test results.

We used specimens from patients who underwent scalpel biopsies and ViziLite screening to determine the sensitivity and specificity of the ViziLite Plus with TBlue system; likewise, we used specimens from patients who underwent scalpel biopsies and VELscope screening to determine the sensitivity and specificity of that device.

We calculated statistical confidence intervals (CIs) on the basis of a f distribution and the exact binomial Clopper-Pearson interval. We analyzed the data with statistical software (Mathematica 6.0.3, Wolfram Research, Champaign, Ill.).

**RESULTS**

One hundred two patients who were examined with ViziLite also underwent a biopsy, and 156 patients who were examined with VELscope also underwent a biopsy. The table shows patients’ demographic data and the locations of the lesions identified in both groups.

**ViziLite group.** Of the 102 participants in the ViziLite group who underwent a biopsy, three had dysplasia (one mild, two moderate) and one had cancer; none of these was detected with the adjunct screening device. Consequently, the sensitivity rate of ViziLite—defined as a measure of the likelihood that a patient with dysplasia or carcinoma found on biopsy will have a positive ViziLite result—was 0 percent (0 of four positive findings) (CI, 0-60.2 percent). The ViziLite findings were...
negative in 74 patients with benign lesions and positive in 24 patients with benign lesions. The specificity rate—defined as a measure of the likelihood that a patient with a benign lesion will have a negative ViziLite result—was 75.5 percent (74 of 98 negative findings) (CI, 66.7-82.8 percent). The positive predictive value—defined as the probability that a positive ViziLite test result would be confirmed by scalpel biopsy—was 0 percent (CI, 0-14.3 percent). The negative predictive value—defined as the probability that a negative ViziLite test result would be confirmed by scalpel biopsy—was 94.8 percent (CI, 89.9-99.9 percent).

**VELscope group.** Of the 156 participants in the VELscope group who underwent a biopsy, 11 had dysplasia and one had cancer, six of which also were detected with VELscope (five dysplasias [two mild, three moderate] and one cancer). The sensitivity rate of VELscope—defined as a measure of the likelihood that a patient with dysplasia or carcinoma will have a positive VELscope result—was 50 percent (six of 12 positive findings) (CI, 21.1-78.9 percent). VELscope findings were negative in 56 patients with benign lesions and positive in 88 patients with benign lesions. The specificity rate—defined as a measure of the likelihood that a patient with a benign lesion will have a negative VELscope result—was 38.9 percent (56 of 144 negative findings) (CI, 30.8-46.9 percent). The positive predictive value of VELscope was 6.4 percent (CI, 2.4-13.4 percent), and the negative predictive value was 90.3 percent (CI, 82.8-97.9 percent).

Neither ViziLite nor VELscope identified any lesions that were not already apparent during the clinical examination with a conventional overhead light alone.

The pathological test results from the independent pathologist were in agreement with those from the hospital pathologists who initially analyzed all of the biopsy specimens.

**DISCUSSION**

This is the first study, to our knowledge, to compare ViziLite and VELscope screening results with histopathologic findings in lesions deemed to be clinically innocuous according to conventional light examination.

The poor sensitivity and poor positive predictive values of these devices (ViziLite, 0 percent; VELscope, 50 percent) have significant implications for dentists and physicians who attempt to rely on these aids to determine whether a lesion is benign or precancerous or cancerous. Our study results show that because of their high false-negative rates, potentially large numbers of precancerous and cancerous lesions will be missed with the ViziLite or VELscope. Consequently, both the clinician and patient will have a false sense of security after a negative ViziLite or VELscope finding is reached because many dysplasias probably will have remained undetected and undiagnosed. These high false-negative rates invariably will lead to a delay in diagnosis, and a potentially greater number of oral cancers will be diagnosed at more advanced stages.

Although some published reports have shown that ViziLite improves the sharpness and brightness of oral lesions, Oh and Laskin concluded that ViziLite produced reflections that made visualization more difficult than that with typical operatory lighting. In another study, Farah and McCullough reported that ViziLite did not discriminate between 55 keratotic, inflammatory, potentially malignant and malignant oral mucosal white lesions with positive ViziLite findings that underwent a scalpel biopsy; these results are in agreement with ours. Furthermore, Kerr and colleagues reported that a significant number of suspicious red lesions, which typically are revealed to be dysplasia or frank carcinoma on biopsy, were not detected with ViziLite.

**TABLE**

Demographic data for patients.

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>PERCENTAGE OF PATIENTS*</th>
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<tbody>
<tr>
<td></td>
<td>ViziLite Plus With TBlue†</td>
</tr>
<tr>
<td>Median Age (Years)</td>
<td>39</td>
</tr>
<tr>
<td>Male:Female Ratio</td>
<td>7.5:1</td>
</tr>
<tr>
<td>Tobacco Use</td>
<td>73</td>
</tr>
<tr>
<td>Location of Lesions</td>
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<tr>
<td>Buccal mucosa</td>
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</tr>
<tr>
<td>Retromolar trigone</td>
<td>14</td>
</tr>
<tr>
<td>Tongue</td>
<td>19</td>
</tr>
<tr>
<td>Alveolar mucosa</td>
<td>7</td>
</tr>
<tr>
<td>Gingiva</td>
<td>4</td>
</tr>
<tr>
<td>Floor of mouth</td>
<td>3</td>
</tr>
<tr>
<td>Palate</td>
<td>3</td>
</tr>
</tbody>
</table>

* Unless otherwise specified.
† ViziLite Plus with TBlue is manufactured by Zila, a division of Tolmar, Fort Collins, Colo.
‡ VELscope is manufactured by LED Dental, White Rock, British Columbia, Canada.
Toluidine blue stain. Epstein and colleagues\textsuperscript{14} assessed clinically suspicious lesions with ViziLite and then with toluidine blue stain before biopsy. They found that ViziLite improved the brightness and/or sharpness of the majority of lesions, but the false-positive rate was high; this was reduced with toluidine blue. Toluidine blue is not available commercially or approved by the FDA for evaluating oral lesions. Furthermore, the toluidine blue swab enclosed with ViziLite Plus with TBlue test kits is not approved by the FDA as a diagnostic tool but is intended to be used only as a marker to highlight a lesion. This is stated clearly in the manufacturer’s instructions: “The marking system is not intended to be used as an indicator of lesions warranting further study, including biopsy.”\textsuperscript{7,15}

We found that ViziLite did not detect any of the dysplasias or cancerous lesions in study participants, regardless of whether they were red or white. Furthermore, in our study, as with other studies, ViziLite did not detect any lesions that the clinician did not detect with an overhead examination light alone.

VELscope is based on a principle that excitation by blue or ultraviolet light will generate tissue autofluorescence that is produced by submucosal collagen, elastin and other naturally occurring fluorophores.\textsuperscript{16} Autofluorescence distinguishes fluorescence of naturally occurring tissue components from artificially introduced fluorescent molecules, such as molecular biomarkers.

The mechanism by which clinicians use VELscope’s tissue autofluorescence to detect epithelial carcinomas may be explained by the fact that hemoglobin strongly absorbs the autofluorescent light produced by collagen and elastin.\textsuperscript{17-19} More specifically, the increased presence of submucosal blood resulting from cancer-induced angiogenesis may result in absorption of collagen- and elastin-produced autofluorescent light and, therefore, the tissue area may appear dark during the VELscope examination.

As our study results show, the VELscope examination failed to detect six of 11 dysplasias. Because angiogenesis generally is associated with severe dysplasia or carcinoma in situ, as well as invasive disease, and is absent in healthy oral epithelium, mild dysplasias and moderate dysplasias,\textsuperscript{20} clinicians should not rely on VELscope to detect precancerous lesions.

Huff and colleagues\textsuperscript{21} reported that the addition of VELscope screening to an oral examination with standard overhead lighting resulted in the discovery of a greater number of oral dysplasias in a general dental practice. However, this study has significant limitations because the authors did not report if VELscope actually detected any new dysplastic lesions or even identified all of the lesions that were detected with standard overhead lighting.

In a proof-of-concept study, Lane and colleagues\textsuperscript{22} reported that VELscope had a 98 percent sensitivity and a 100 percent specificity when discriminating between carcinoma in situ or invasive cancer and healthy oral mucosa. However, in their study, unlike ours, the investigators used VELscope only in patients who were already known to have carcinoma in situ or invasive cancer on biopsy. Furthermore, all abnormalities found with VELscope also were observed with the standard examination light alone. Like Lane and colleagues, we did not find any lesions with VELscope that were not apparent during an oral examination involving the use of a standard overhead light.

The increased submucosal hemoglobin that apparently has been detected with VELscope can result from a variety of traumatic and inflammatory conditions, which may account for VELscope’s poor specificity. The high false-positive rate associated with VELscope has raised concerns about its potential harm, causing unnecessary stress and fear among patients, as well as increasing morbidity through unnecessary surgical biopsy procedures.\textsuperscript{23}

Manufacturer’s advice. The manufacturer of VELscope offers advice to reduce the number of false-positive results caused by inflammation and other noncancerous lesions that may result from the presence of submucosal blood.\textsuperscript{24} The company recommends applying pressure to a lesion that appears dark and, therefore, is suspicious to see if it blanches (that is, if the green color returns with pressure). This advice appears problematic because absorption of autofluorescent light resulting from true angiogenesis also may be hidden by this temporary tourniquet action, while absorption of autofluorescent light from the presence of submucosal blood caused by minor trauma may not. Indeed, there is no clinical basis to support this procedure based on our review of the VELscope literature.

Study limitations. Experienced clinicians performed the clinical examinations and examinations with the adjunct screening aids. However, they were not calibrated in using the VELscope and ViziLite devices; they also were not cali-
brated regarding classification of lesions identified during the oral examinations. The two adjunct screening aids are fairly straightforward in their application, so the lack of calibration was not as important as the lack of calibration in classifying lesions. Clinicians should keep this fact in mind when interpreting our results. We made no attempt to assess variability between observers.

Regarding other study limitations, it is worth noting that because this was not an opportunistic screening, in which dentists might use these devices for all adult patients regardless of whether or not they have a visible lesion, our study design does not reflect exactly the way in which these devices are currently used. Again, clinicians should keep this fact in mind when interpreting these results. Because specialists from different fields conducted the examinations in this study, day-to-day practice and use of these adjunct screening aids may differ.

**CONCLUSION**

Although ViziLite and VELscope have been promoted as valuable adjuncts in the early detection of oral precancerous and cancerous lesions, the results of our study indicate that they do not add any benefits to a conventional screening examination involving the use of a standard overhead light. Additional clinical studies are needed to evaluate the effectiveness and costs of light-based oral cancer screening aids before they can be recommended.

**Disclosure.** None of the authors reported any disclosures.

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Evaluation of an autofluorescence based imaging system (VELscope™) in the detection of oral potentially malignant disorders and benign keratoses

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S U M M A R Y
Early detection of oral cancer is crucial in improving survival rate. Identification and detection of oral potentially malignant disorders (OPMD) allow delivery of interventions to reduce the evolution of these disorders to malignancy. A variety of new and emerging diagnostic aids and adjunctive techniques are currently available to potentially assist in the detection of OPMD. The objective of the present study was to evaluate the accuracy of autofluorescence against conventional oral examination and surgical biopsy.

A total of 126 patients, 70 males and 56 females (mean age 58.5 ± 11.9 years) who presented to the Oral Medicine Clinics at King’s and Guy’s Hospitals, London with oral white and red patches suspicious of OPMD were enrolled. Following a complete visual and autofluorescence examination, all underwent an incisional biopsy for histopathological assessment.

Seventy patients had oral leukoplakia/erythroplakia, 32 had oral lichen planus, 9 chronic hyperplastic candidiasis and rest frictional keratosis (13) or oral submucous fibrosis (2). Of 126 lesions, 105 (83%) showed loss of fluorescence. Following biopsy 44 had oral epithelial dysplasia (29 mild, 8 moderate and 7 severe). The sensitivity (se) and specificity (sp) of autofluorescence for the detection of a dysplastic lesion was 84.1% and 15.3% respectively.

While VELscope was useful in confirming the presence of oral leukoplakia and erythroplakia and other oral mucosal disorders, the device was unable to discriminate high-risk from low-risk lesions.

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I n t r o d u c t i o n

Oral cancer is a growing problem in many European countries including the United Kingdom.¹ Delays in diagnosis are frequently reported² either due to poor symptom recognition³ or missed diagnosis.⁴ The UK guidelines for the early diagnosis of Head & Neck cancers were published in 2005 setting out criteria for urgent referral for suspicious lesions⁵ and the British Dental Association and FDI recommend that systematic visual screening examination should be carried out on every patient at the beginning of a new course of treatment. While detection of asymptomatic cancers could be a problem in dental practices due to poor attendance of high-risk patients⁶, oral potentially malignant disorders⁷ (OPMD) provide a long preclinical phase during which high-risk patients could be identified to provide interventions.

Opportunistic screening by visual clinical examination at dental practices will identify OPMD and other mucosal disorders with similar clinical presentations.⁸ It is estimated that up to 15% of the population have oral mucosal diseases at any one time, but only very few have the characteristics of OPMD.⁹ It is therefore a problem for practitioners to identify and refer OPMD with confidence. Several chair-side adjunctive aids have been developed to help practitioners with oral cancer screening with the aim of diagnosing high-risk lesions. None of these have been tested adequately in primary care settings.¹⁰

Autofluorescence is one potential technique that may be used to facilitate the visualisation and management of oral cancer and OPMD. As early as in 1924, it was observed that the autofluorescence of tissues could potentially be used for cancer detection.¹¹ Autofluorescence works on the principle that certain biofluorphores present within the tissue become fluorescent on excitation with a suitable wavelength (400–460 nm) light source. However, diseased tissues lose fluorescence (fluorescence visualisation loss – FVL) due to disruption in the distribution of these biofluorphores, and appear darker in colour.
The aim of this study was to evaluate the accuracy of autofluorescence examination in its ability to delineate high-risk oral mucosal lesions from other lesions already diagnosed by a specialist, to allow estimates of sensitivity and specificity of the technique.

Materials and methods

One hundred and sixty-four consecutive patients aged over 16 years presenting in oral medicine clinics at two London Hospitals with white, red and mixed white and red patches were invited to participate in the study. One hundred and twenty-six patients (76.8%) consented and were investigated by a standard protocol that involved clinical visual examination and autofluorescence examination followed by biopsy. The study was approved by Institutional Research and Ethics Committees (08/H0808/20).

Following a comprehensive clinical examination under an incandescent light source the clinical diagnosis was established by the operator (KHA) and validated by a second experienced examiner (SW). The principal area (site) of morphologically altered mucosa was selected excluding any ulcerated areas (by consensus of both examiners) and photographed. All further investigations were performed on this clinically detected area of mucosal abnormality. Autofluorescence examination was performed using the VELscope™ (Visually Enhance Lesion Scope) under dimmed room light, with protective eye wear worn by the patient throughout the procedure. The possible outcome of the autofluorescence examination was determined by the manufacturer’s literature i.e. FVL – fluorescence visualization loss, FVR – fluorescence visualization retained and FVI – fluorescence visualization increased. Both examiners were calibrated by an experienced professional from the LED Diagnostics (the manufacturer).

A surgical biopsy was performed for histopathological assessment and the selection of the biopsy site took into consideration any area of FVL identified by the VELscope within the lesion. The presence or absence of dysplasia in the biopsy specimen was recorded by an experienced oral pathologist (PRM).

Data collected was entered through the IBM SPSS 18 (Statistical Package for the Social Sciences). Sensitivity and specificity of the autofluorescence test results, compared to clinical diagnosis by a specialist and dysplasia grade from biopsy, were calculated. Differences and associations between the autofluorescence test and dysplasia grade were examined using either Fisher’s exact test or 

\[ \chi^2 \]  

test with significance set at \( P < 0.05 \). All tests were two-sided. A receiver operating characteristic (ROC) curve was used to estimate the diagnostic value of the test.

Results

The profile of 126 patients enrolled in this study is given in Table 1. Of 126 lesions, more than half \( (n = 70) \) were clinically diagnosed as either leukoplakia or erythroplakia. Thirteen lesions were clinically diagnosed as frictional keratoses and 32 as oral lichen planus or lichenoid reaction. The remaining lesions consisted of 9 chronic hyperplastic candidiasis and 2 oral submucous fibrosis. One hundred and sixteen patients underwent surgical biopsy from which oral epithelial dysplasia was confirmed in 44 patients.

Autofluorescence examination was performed on all 126 patients. One hundred and five lesions showed FVL \((83.3\%)\) whereas 16 retained the fluorescence \((12.7\%)\) and appeared apple green in colour. Three of the lesions showed increased fluorescence and 2 had a mixed result showing both loss and increased fluorescence in different areas within the lesion. Of 105 FVL cases, more than 50% \((n = 53)\) showed complete loss of fluorescence whereas 29 showed partial loss of fluorescence. In 23 lesions, FVL extended beyond the clinically evident oral lesion.

Leuko/erythroplakia vs. other group

Of 70 leuko/erythroplakia cases, 61 \( (87.1\%) \) showed FVL whereas only 9 \( (12.9\%) \) that appeared clinically white had a negative test result (Table 2). All 9 cases of erythroplakias showed FVL. In the case of other oral diagnostic categories, 44 \( (78.6\%) \) showed FVL with the remaining 12 \( (21.4\%) \) showing a negative test outcome. In particular, among the 13 frictional keratoses cases, 9 \( (69.2\%) \) showed FVL. Autofluorescence examination showed a sensitivity and specificity of 87.1% and 21.4%, respectively. Positivity of autofluorescence (FVL) for the leuko/erythroplakia group was not significantly different compared with the other group \( (\chi^2 = 1.65, P = 0.23) \). ROC curve for autofluorescence as a tool to detect leuko/erythroplakias showed a poor diagnostic value \( (AUC = 0.52, 95\% CI: 0.42–0.62, P = 0.72) \).

Dysplasia group

Autofluorescence showed a sensitivity of 84.1% as 37 out of 44 lesions with dysplasia recorded as FVL in contrast to only 7 which did not show any FVL. Among the 7 FVL negative dysplasias, 5 were graded mild and 2 as moderate. But the autofluorescence was not highly specific for dysplastic oral lesions as FVL was observed in 61 \( (84.7\%) \) of the non-dysplastic oral lesions, leading to a low specificity \( (15.3\%) \). No significant difference was noted among the dysplasia group in relation to the autofluorescence test results \( (\chi^2 = 0.00, P = 1.00) \). ROC curve for autofluorescence as a tool for the detection of dysplasia group also showed a poor diagnostic value \( (AUC = 0.49, 95\% CI: 0.39–0.61, P = 0.96) \).

Discussion

Five-year survival rates for oral cancer have not changed for several decades. Poor survival is at least in part due to the failure in early detection of OPMD and oral cancers. To this end improving diagnostic abilities of primary care dentists/physicians and also

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<thead>
<tr>
<th>Lesion site</th>
<th>All ( n = 126 )</th>
<th>Leuko/erythroplakia ( n = 70 )</th>
<th>Dysplasia ( n = 44 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buccal mucosa</td>
<td>54</td>
<td>21</td>
<td>10</td>
</tr>
<tr>
<td>Tongue</td>
<td>40</td>
<td>21</td>
<td>14</td>
</tr>
<tr>
<td>Floor of mouth</td>
<td>11</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Palate</td>
<td>11</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>Alveolar ridge</td>
<td>7</td>
<td>6</td>
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</tr>
<tr>
<td>Female</td>
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<td>24</td>
<td>18</td>
</tr>
<tr>
<td>Ethnicity</td>
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<tr>
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<td>76</td>
<td>51</td>
<td>28</td>
</tr>
<tr>
<td>Non-white</td>
<td>50</td>
<td>19</td>
<td>16</td>
</tr>
<tr>
<td>Tobacco history</td>
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<td></td>
</tr>
<tr>
<td>Current smokers</td>
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<td>41</td>
<td>24</td>
</tr>
<tr>
<td>Ex-smokers</td>
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<td>15</td>
<td>13</td>
</tr>
<tr>
<td>Never smoked</td>
<td>37</td>
<td>24</td>
<td>7</td>
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<tr>
<td>Alcohol history</td>
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</tr>
<tr>
<td>Current users</td>
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<td>57</td>
<td>33</td>
</tr>
<tr>
<td>Ex-users</td>
<td>8</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Never used</td>
<td>26</td>
<td>10</td>
<td>6</td>
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</tbody>
</table>
facilitating less interventional investigations in secondary care units remain important cornerstones in the research agenda. We investigated the utility of autofluorescence as a diagnostic test to evaluate its accuracy in the detection of oral leuko/erythroplakia and oral epithelial dysplasia.

FVL was observed in the majority (87.1%) of the clinically diagnosed cases of leuko/erythroplakias, lesions that carry a relatively higher risk of malignant transformation compared to other OPMD. More interestingly, FVL was positive in all 9 cases of erythroplakias giving a sensitivity of 100%. FVL was also observed in the majority of the cases (84.1%) that were histopathologically diagnosed as dysplasia and notably the VELscope detected all severe dysplasia cases (n = 9). These results notably demonstrate the ability of the technique to detect high-risk lesions. However, it was disappointing to note that autofluorescence examination was positive in majority of the other white/red lesions that to a non-specialist could resemble leuko/erythroplakia. This finding re-affirms the lack of specificity of the technique for the detection of leuko/erythroplakia. In addition, VELscope was also unable to detect 7 dysplasias (5 mild, 2 moderate), thus further undermining the utility of the device, if the objective is to pick all dysplasia cases. Limitations of autofluorescence examination in discriminating between dysplasia and non-dysplasia cases have been reported in a recent study where 6 cases of dysplasia were not detected by the VELscope.

Comparison of the results of the present study with published data proved to be difficult due to limited number of studies in the literature reporting sensitivity and specificity of the device. Only one previous study appears to have employed autofluorescence in a systemic examination on a cohort of patients. The study was conducted at the British Columbia Cancer Agency (BCCA) where a prototype of the VELscope was investigated by the group. Using the blue-excitation light, 50 lesions were examined which included 33 oral cancers, 11 severe dysplasia and carcinoma-in-situ and 6 with no oral mucosal lesions. The authors reported a sensitivity of 98% and specificity of 100% against the gold standard (histology). Our data show a low specificity (15.3%) in discriminating high-risk (dysplasias) from benign lesions. Further well designed studies are needed to examine the role of VELscope as an oral examination system in primary care.

### Conclusion

In conclusion, our study demonstrated a relatively high sensitivity (84%) and a low specificity (15%) in discriminating high-risk (dysplasias) from benign lesions. Further well designed studies are needed to examine the role of VELscope as an oral examination system in primary care.
Conflict of interest statement

We thank Dr. Connie Yang for assistance in setting up the data entry system and Dr. Derek Cooper for the data analysis. VELscope system for the study was supplied by LED Diagnostics.

References

Auto-fluorescence imaging of potentially malignant mucosa lesions

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UNIVERSITY OF COLOGNE

Objective and study design. Several noninvasive adjunctive methods have been proposed for identification of potentially malignant mucosa lesions. The VELscope is an optical device for detecting spatial changes in mucosa autofluorescence caused by premalignant lesions in conjunction with an intraoral exam. The aim of our prospective study was to correlate loss of autofluorescence from undiagnosed mucosa lesions with histology.

Results. In total 64 patients considered at risk for squamous cell carcinoma (20 had previous OSCC) were included in the study. Regions with fluorescence visualization loss were considered as malignant or dysplastic. All patients underwent biopsy after VELscope examination. In 22 patients (34.4%) a loss of autofluorescence indicating a squamous intraepithelial neoplasia (SIN) or malignant mucosal lesion was detected. The sensitivity of identification of malignant and dysplastic areas with the VELscope was 100% and the specificity was 80.8%, respectively, compared with histology as gold standard. The positive predictive value was 54.5% and the negative predictive value was 100% respectively.

Conclusion. Evaluation of autofluorescence imaging with VELscope can assist in the identification of malignant and potentially malignant oral lesions from normal mucosa in high-risk patients but does not help discriminating benign lesions from malignant or premalignant mucosal conditions. (Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;111:568-577)
eral clinical features have been identified. It has been shown that lesions in patients older than 70 years with long-lasting history have an increased risk of becoming malignant.\textsuperscript{3,10} The location in the oral cavity also has an impact on malignant transformation: lesions located in the floor of mouth and the tongue show the highest rates of malignant conversion in contrast to lesions located at the hard palate and cheek.\textsuperscript{11}

To improve follow-up in patients with leukoplakia, as well as effectively screen high-risk patients, additional noninvasive techniques have been proposed (Table I).\textsuperscript{12-19} Especially cytopathology-based techniques with optional computer-assisted analysis as well as analysis of extracellular matrix proteins or allelic imbalances showed promising results in recent studies, but sufficient data are missing for low-risk populations.\textsuperscript{20-22} Vital-staining procedures using toluidine blue have been performed to identify lesions with rapid cell division, which occur in dysplastic lesions and invasive carcinoma. Selective staining of malignant cells is the result of an increased amount of nucleic acids and more intracellular canals, which improve penetration of the dye into the neoplastic cells.\textsuperscript{23} In the meta-analysis of Rosenberg and Cretin,\textsuperscript{24} the sensitivity of toluidine blue–positive staining in a high-risk population ranged from 93.5% to 97.8% and the specificity from 73.3% to 92.9%, respectively. The sensitivity reported for vital staining of suspicious mucosa lesions with toluidine blue was slightly increased in comparison with visual inspection, but the prognostic significance was limited by the increased rate of false positive testings.\textsuperscript{19,24-26} In the United States and Europe, toluidine blue is available as part of the Vizilite plus product (chemiluminescent detection of suspicious mucosa lesions after treatment with acetic acid; Zila-Dental Inc., Fort Collins, CO, USA) for further evaluation and monitoring of lesions by providing physical marking of lesions already differently identified with Vizilite. A recently published study on suspicious oral lesions revealed a significant reduction of false positive results by combining the chemiluminescent examination with toluidine blue staining.\textsuperscript{13} Because prospective studies have been carried out only in high-risk populations, the value of toluidine blue staining as a screening test has been questioned.\textsuperscript{27} In recent years, autofluorescence imaging has gained interest in clinical practice for noninvasive imaging of the oral mucosa. Autofluorescence in the oral cavity is achieved by different excitation wavelengths and arises from naturally occurring fluorochromes that are located in the epithelial cell lining and submucosa (e.g., collagen, elastin, keratin, oxidized flavine adenine dinucleotide (FAD), and nicotinamide adenine dinucleotide [NADH]).\textsuperscript{28} All of the previously mentioned fluorochromes show fluorescence in the green spectral range when excited between 375 and 440 nm.\textsuperscript{29} The normal intrinsic pattern of autofluorescence is modified by absorption and scattering. Regarding the different ana-

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|l|l|l|l|l|}
\hline
Author et al., 1995\textsuperscript{14} & Oral examination & 3 carcinoma, 18 leukoplakia, 31 lichen planus, 2 lupus erythematosus & 2027 & 74% & 99% & 67% & 99% \\
\hline
Sciubba, 1999\textsuperscript{18} & Oral CDx & 131 malignant or dysplastic lesions, 196 benign lesions, 618 no biopsy & 945 & 100% & 93% & 90% & 100% \\
\hline
Poate et al., 2004\textsuperscript{16} & Oral CDx & 7 carcinoma, 14 dysplasia, 28 benign lesions, 63 no biopsy & 112\textsuperscript{a} & 71% & 32% & 44% & 60% \\
\hline
Remmerbach et al., 2004\textsuperscript{17} & Cytology and DNA-cytometry & 92 carcinoma, 93 leukoplakia, 142 benign lesions & 332\textsuperscript{b} & 98% & 100% & 100% & 98.1% \\
\hline
Driemel et al., 2007\textsuperscript{12} & Tenascin-C in brush biopsies & 60 carcinoma, 56 leukoplakia, 28 inflammatory lesions, 15 normal mucosa & 159 & 93% & 83% & 88% & 90% \\
\hline
Warnakulasuriya and Johnson, 1996\textsuperscript{19} & 1% toluidine blue staining & 18 carcinoma, 39 dysplasia, 29 keratotic/hyperplastic lesions, 58 no biopsy & 145\textsuperscript{c} & 82% & 46% & 81% & 64% \\
\hline
Epstein et al., 2008\textsuperscript{13} & ViziLite plus with toluidine blue staining & 9 carcinoma, 45 SIN, 43 benign lesions & 97\textsuperscript{d} & 100% & 55% & 37% & 100% \\
\hline
Lane et al., 2006\textsuperscript{15} & VELscope & 33 carcinoma, 11 SIN, 6 normal mucosa & 50 & 98% & 100% & 100% & 86% \\
\hline
\end{tabular}
\caption{Screening methods for detection or oral cancer and precancer}
\end{table}

\textsuperscript{a}Eight patients were excluded from the study because of insufficient material and assigned for incisional biopsy.

\textsuperscript{b}In total, 332 cytological diagnoses were obtained in 205 patients.

\textsuperscript{c}In total, 145 lesions were analyzed in 102 patients.

\textsuperscript{d}Eighty-four patients with 97 lesions were analyzed.
tomical regions in the oral cavity, almost identical autofluorescence spectra have been detected in healthy individuals using different excitation wavelengths. However, the dorsum of the tongue and the vermilion border of the lips showed different autofluorescence characteristics that were caused by endogenous porphyrins, produced by epithelial cells or microorganisms at the dorsum of the tongue.29,30 According to the literature, autofluorescence spectroscopy is capable of differentiating malignant tumors from healthy oral tissue with a sensitivity and specificity higher than 95%. Several studies revealed an improved sensitivity and specificity for malignant lesions by adding autofluorescence imaging to conventional clinical examination in different anatomic regions; however, one study showed a lower sensitivity for autofluorescence than for white light examination.31-33

In July 2006, an easy-to-use handheld device called VELscope (Visually Enhanced Lesion scope; LED Dental Inc., Burnaby, BC, Canada) for autofluorescence imaging became commercially available for screening of the oral mucosa. The device was intended to enhance the identification and visualization of premalignant and malignant oral lesions that may not be visible to the naked eye. Until now, only a limited number of case reports and observational studies evaluating the use of the VELscope have been published (Table II).15,34-36 The objective of this study was to evaluate fluorescence loss with the VELscope device to determine its ability to differentiate between benign, dysplastic and neoplastic oral mucosal lesions before histologic confirmation.

### MATERIAL AND METHODS

#### Patients

Oral and VELscope examinations were performed on 64 patients referred to the Department of Oral and Cranio-maxillo-facial Surgery to rule out invasive squamous cell carcinoma. Written consent was obtained from all patients. Patients with advanced squamous cell carcinomas were excluded. The Institutional Review Board of the University of Cologne approved this prospective study.

Photo documentation was carried out with a Canon EOS 20 D SLR, equipped with a Canon 100-mm Macro Lens and Macro Ring Lite (Canon MR-14 EX; Canon Inc., Tokyo, Japan). For all white-light pictures, the same parameters for ISO, exposure time, and aperture were used (ISO 100, 1/125 s, f 22). For correct visualization and documentation of autofluorescence patterns, the examination room was darkened. All lesions underwent histopathological evaluation by a trained pathologist (U.D.) after examination with the VELscope to evaluate the prognostic power of this new testing system.37 All specimens were placed in 4% buffered formalin for fixation.

#### VELscope device

The VELscope device was purchased from MECTRON the European distributor for LED, Vancouver, Canada. For evaluation of the suspicious lesions, the room was shaded and the hand piece was covered with a lens cover. A metal-halide lamp for detection of abnormal epithelial and stroma structure produced blue light between 400 and 460 nm. The excitation blue light was projected on the oral mucosa by a dichroic mirror. Through the back of the hand piece, tissue autofluorescence above 480 nm could be identified as green light. For documentation purposes, the above-mentioned camera equipment without the ring flash was mounted on the back of the hand piece using an adapter (PhotoMed Velscope Photography System, Photomed International, Van Nuys, CA). Because of the low amount of light coming through the hand piece, adjustment of camera setting was mandatory (ISO 1600, 1/60 s, f 5). The magnification factor as well as angle of documentation were adjusted to the clinical picture so as to allow correct identification of altered autofluorescence patterns. According to the literature, the complete loss of

### Table II. Studies published with the VELscope device

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Study design</th>
<th>N=</th>
<th>Results</th>
</tr>
</thead>
</table>
| Lane et al., 200615 | Observational study | 50 follow-up patients | 98% sensitivity  
6 normal mucosa  
11 dysplasias/CIS  
33 OSCCs  
All carcinomas were associated with an FVL.  
91% of dysplastic lesions and 100% of OSCCs revealed an FVL.  |
| Poh et al., 200636 | Observational study | 20 patients with biopsy-proven OSCC | In 19 of 20 cases the area of FVL was increased by 10.3 ± 5.7 mm in contrast to conventional white light image. |
| Poh et al., 200635 | Case report | 3 follow-up patients | Biopsy revealed invasive carcinomas in 2 cases and dysplastic lesion in 1 case. |

CIS, carcinoma in situ; FVL, fluorescence loss; OSCC, oral squamous cell carcinoma.
were excised completely. Biopsies were taken from well as moderate and severe dysplasia (SIN II and III) after the VELscope examination. Carcinoma in-situ as history of oral cancer. Table III. Twenty patients (31.2%) had a previous logic characteristics of the patients are presented in 59.8 years were included in our study. Clinicopatho-
tients (25 female and 39 male) with an average age of

RESULTS

Biopsies were performed in all lesions examined after the VELscope examination. Carcinoma in-situ as well as moderate and severe dysplasia (SIN II and III) were excised completely. Biopsies were taken from the area of fluorescence loss, if applicable. The histopathologic evaluation revealed benign lesions consisting of lichen planus, hyperkeratosis, chronic inflammation, and so forth in 52 patients (81.2%). In 4 cases SINs were reported. One case (1.6%) of SIN occurred in a patient who had previously treated for invasive carcinoma of the buccal mucosa.

Invasive oral squamous cell carcinoma was diagnosed in 8 patients (12.5%). Two patients (3.1%) with invasive carcinomas had been treated for invasive carcinoma of the mandible and floor of mouth, respectively. In 6 cases, new carcinoma was diagnosed in the retromolar region, floor of mouth, buccal mucosa (n = 2), lateral border of the tongue, and the mandible. In all patients diagnosed with invasive oral carcinoma, stagg-
ing and curative-intended resection were performed.

DISCUSSION

In our prospective single-center study, a series of patients with undiagnosed mucosal lesions were ana-
yzed by clinical examination, autofluorescence evaluation using the VELscope, and histopathological con-
firmation. The statistical analysis confirmed a limited specificity (80.8%) and high sensitivity (100%). How-
ever, it should be kept in mind that the data were obtained from a high-risk group, i.e., 20 patients had been treated for oral cancer before. Consequently, the results cannot be assigned to a general population. The main cause for the limited specificity was the rate of false positive results (15.6%). The threshold between fluorescence loss and diminished fluorescence is arbitrary and related to the experience of the user, which is one of the major shortcomings of the VELscope, as this is very subjective. The high rate of false positive ratings limits the positive predictive (54.5%) value, as well.

Statistical analysis

The sensitivity, specificity, and positive and negative predictive values for the VELscope examination were calculated from a contingency table. The $\chi^2$ test ($P < .05$) was used for additional analysis for categorical variables and relationships in the contingency table. In detail, the $\chi^2$ test was used to test autofluorescence properties (FVL yes or no) versus the histologic results (benign lesions or squamous intraepithelial neoplasm [SIN]/carcinoma), gender and localization of the lesion. The sensitivity measured the proportion of malign and dysplastic lesions (carcinoma and SIN) that were correctly identified by autofluorescence loss with the VELscope. The specificity was the proportion of benign lesions that were correctly identified. The positive predictive value (PPV) was the proportion of patients with positive VELscope results who were correctly diagnosed by histopathology. The PPV was calculated as the number of true positive results of the VELscope (SIN and carcinoma) divided by the number of true positive and the number of false positive cases. The negative predictive value (NPV) was the proportion of patients with negative VELscope results who were correctly diagnosed as negative by histology (benign lesions). The NPV was calculated as number of true negative cases divided by the number of true negative cases added by the false negative cases.

The negative predictive value (NPV) was calculated as number of true negative cases divided by the number of true negative cases added by the false negative cases.

Invasive oral squamous cell carcinoma was diagnosed in 8 patients (12.5%). Two patients (3.1%) with invasive carcinomas had been treated for invasive carcinoma of the mandible and floor of mouth, respectively. In 6 cases, new carcinoma was diagnosed in the retromolar region, floor of mouth, buccal mucosa (n = 2), lateral border of the tongue, and the mandible. In all patients diagnosed with invasive oral carcinoma, stagg-
ing and curative-intended resection were performed.

The autofluorescence characteristics ranged from slightly decreased green fluorescence to increased flu-
orescence with orange glow caused by bacterial por-
phyrins (Figs. 1 and 2). In 42 (65.6%) cases the VELscope revealed no FVL and the histologic report showed a benign lesion. An FVL indicating malignant changes or SIN when the histologic results were a benign lesion (false positive), occurred in 10 patients (15.6%) (Fig. 3). However, VELscope evaluation identi-
fied all dysplastic and invasive carcinoma by fluores-
ce loss (Fig. 4). No lesion was rated as false nega-
tive in our series (Table III). Sensitivity and specificity for the autofluorescence evaluation with the VELscope identifying SIN or carcinoma were 100.0% and 80.8%, respectively. The positive and negative predictive values were 54.5% and 100%, respectively. Because of the 10 patients with fluorescence loss and benign histology report, the false positive rate was 15.6%. No statistical association between gender and localization of lesions versus fluorescence loss could be shown ($P = .281$, $P = .744$, respectively). However, the clinical appearance and loss of fluorescence pattern correlated with red and ulcerous lesions ($P < .001$). Also, fluorescence loss (FVL) was significantly associated with SIN or invasive carcinoma compared with that of normal tissue ($P < .0001$, Pearson $\chi^2$ test).
For clinical purposes, it would not be acceptable to refer almost 50% of patients for a biopsy that was not necessary. In comparison with other adjunctive methods (Table I) the sensitivity in identifying SIN, as well as malignant lesions by autofluorescence, attained high values. However, the specificity in our small series was higher than the values reported from other studies with toluidine blue and Vizilite (Table I).13,16,19

Comparing our results with the literature, there is only one study that focused on fluorescence intensity in head and neck cancer patients. Kulapaditharom et al.33 studied 67 patients with highly suspicious lesions located in the head and neck area with laser-induced fluorescence endoscopy (LIFE) before biopsy. The results showed similar values for sensitivity and specificity, respectively (92.86% and 78.57%, respectively). The authors concluded that LIFE identified cancerous and dysplastic lesions more reliably than white-light examination alone. However, the small field of view and the decreased depth of field were considered as disadvantages of this technique.

In a series of 33 former OSCC patients, Lane et al.15 reported a specificity of 100% with the VELscope. Although the number of patients included in Lane et al.’s study15 and our series were similar, our series contained miscellaneous histologic diagnoses. Hence, it can be concluded that our cohort, as well as Lane et al.’s study,15 consisted of former oral cancer patients with an increased risk for mucosal abnormalities compared with the general population. However, the aim of our study was to obtain autofluorescence data on a variety of histologic distinct lesions so as to evaluate the discriminatory power of autofluorescence examination with the VELscope.

In our series, all carcinomas (n = 8) and SINs (n = 4) were identified properly with the VELscope, but in

### Table III. Characteristics of lesions, including histologic and autofluorescence results

<table>
<thead>
<tr>
<th>Patients</th>
<th>Total, n (%)</th>
<th>History of OSCC, n (%)</th>
<th>Fluorescence retained, n (%)</th>
<th>Fluorescence loss (FVL), n (%)</th>
<th>Statistical analysis (chi-square test)</th>
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<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Female</td>
<td>25 (39.1)</td>
<td>7 (10.9)</td>
<td>14 (21.9)</td>
<td>11 (17.2)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>39 (60.9)</td>
<td>13 (20.3)</td>
<td>28 (43.7)</td>
<td>11 (17.2)</td>
<td></td>
</tr>
<tr>
<td>Localization</td>
<td></td>
<td></td>
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<tr>
<td>Floor of mouth</td>
<td>10 (15.6)</td>
<td>5 (7.8)</td>
<td>7 (10.9)</td>
<td>3 (4.7)</td>
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<td>Palate</td>
<td>7 (10.9)</td>
<td>1 (1.6)</td>
<td>5 (7.8)</td>
<td>2 (3.1)</td>
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<td>Alveolar process</td>
<td>12 (18.8)</td>
<td>5 (7.8)</td>
<td>6 (9.4)</td>
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<tr>
<td>Tongue</td>
<td>13 (20.3)</td>
<td>5 (7.8)</td>
<td>8 (12.5)</td>
<td>5 (7.8)</td>
<td></td>
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<tr>
<td>Buccal mucosa</td>
<td>22 (34.4)</td>
<td>4 (6.3)</td>
<td>16 (25)</td>
<td>6 (9.4)</td>
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<td>Lesion appearance</td>
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<td>Red</td>
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<td>3 (4.6)</td>
<td>2 (3.1)</td>
<td>5 (7.8)</td>
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<td>10 (15.6)</td>
<td>30 (47)</td>
<td>3 (4.6)</td>
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<td>Mixed</td>
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<td>2 (3.1)</td>
<td>0</td>
<td>4 (6.3)</td>
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<td>Histology</td>
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<td></td>
<td></td>
<td></td>
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<td>Benign lesions</td>
<td></td>
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<tr>
<td>Lichen planus</td>
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<td>1 (1.6)</td>
<td>2 (3.1)</td>
<td>4 (6.3)</td>
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<tr>
<td>Hyperkeratosis/acanthosis</td>
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<td>13 (20.3)</td>
<td>36 (56.2)</td>
<td>4 (6.3)</td>
<td></td>
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<tr>
<td>Amalgam tattoo</td>
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<td>1 (1.6)</td>
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<td>Granulation tissue</td>
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<td>0</td>
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<td>Chronic inflammation</td>
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<td>3 (4.6)</td>
<td>2 (3.1)</td>
<td>1 (1.6)</td>
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<td>Fibroma</td>
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<tr>
<td>SIN</td>
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<td>1 (1.6)</td>
<td>0</td>
<td>4 (6.3)</td>
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<td>SIN II</td>
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<td>SIN III</td>
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<td>Carcinoma</td>
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<td>2 (3.1)</td>
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<td>G1</td>
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<td>G2</td>
<td>5 (7.8)</td>
<td>2 (3.1)</td>
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<tr>
<td>G3</td>
<td>1 (1.6)</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

OSCC, oral squamous cell carcinoma.

*Statistically significant values (P < .05) for chi-squared test.
the group with nonmalignant lesions, a fluorescence loss was noted in inflammatory lesions too. In our series, 4 patients (6.3%) with lichen planus as well as 4 patients with hyperkeratosis/acanthosis (6.3%) showed fluorescence loss. In 2 other patients, granulation tissue and chronic inflammation were associated with an autofluorescence loss. Regarding the clinical appearance of the lesions studied in our series, red as well as ulcerous lesions were statistically associated with fluorescence loss. This “false positive” result can be explained by the increased subepithelial blood flow and altered metabolic activity in inflamed mucosa.39 This observation was reported by another study using the VELscope. Owing to the conflicting results, the author did not recommend the VELscope for oral cancer screening purposes.43 Subepithelial vessels in the floor of mouth cause a loss of fluorescence in normal mucosa (Fig. 5). To reduce the rate of false positive results,
Kois and Truelove proposed a follow-up visit 2 weeks after the initial examination under the assumption that acute inflammatory lesions should have resolved by then. Because a subset of oral squamous cell carcinomas can arise on inflammatory lesions, a biopsy is mandatory if the lesion has not resolved. Additionally, soft pressure with a clear tongue depressor could be used to reduce blood flow in inflammatory lesions and restore normal autofluorescence, in contrast to malignant or premalignant lesions where the fluorescence loss is not altered by the same maneuver. To eliminate artifacts caused by blood clots and brush biopsies, the VELscope examination should be performed before any mechanical alteration of the lesion because hemoglobin can cause a false positive fluorescence loss.

In our study, all lesions were analyzed histologically so as not to miss any malignant lesion. Because the VELscope is an adjunctive method only, it is recommended to perform a biopsy if clinical judgment clas-
sifies the mucosa alteration as suspicious. According to our 3-year experience with the VELscope system, reproducible thresholds between reduced autofluorescence and a complete loss of fluorescence can be ascertained. Actually, this subjective classification depends on the experience and knowledge of the investigator. A quantification of fluorescence loss, for example expressed as a ratio to surrounding normal tissue or analysis of the emitted spectrum of light, would be helpful to assist in characterization of the lesions. In a current study, our group is working on a classification system for digital VELscope pictures using commercially available software for image analysis.46 Because only one experienced oral and maxillofacial surgeon without the knowledge of the histologic result rated all examinations, no value for interobserver variability can be applied. Keeping in mind the subjective judgment of fluorescence loss, it is recommended that several experienced investigators rate the autofluorescence pattern in a blinded fashion.

In our series, 3 histologically proven verrucous leukoplakias without any signs of dysplasia were assessed with the VELscope. Because of an increased amount of keratin, these lesions presented a gain of autofluorescence (Fig. 2). Because no verrucous carcinoma was included in our series, it can be assumed that only invasive verrucous carcinoma would reveal a similar gain in autofluorescence as verrucous leukoplakia. Hence, in verrucous lesions the autofluorescence criteria (i.e., loss of fluorescence) for suspecting malignant conversion cannot be applied. The data compiled from the literature revealed no specific criteria for autofluorescence assessment of verrucous lesions either. Only one study that analyzed the autofluorescence pattern of different mucosa lesions by means of artificial neural networking revealed no correlation between spectral patterns and verrucous lesions.47 Consequently, further histology-controlled studies are required to correlate the autofluorescence pattern in verrucous lesions. The porphyrinlike fluorescence was noted in all 3 verrucous lesions in our series. According to the literature, this red or orange fluorescence is caused by bacteria and is not specific for malignant conversion.29

Although our study included a small number of selected patients, the variety of histologically confirmed diagnoses supported the conclusion that autofluorescence imaging can assist in the complex algorithm of diagnosing oral cancer. However, the identification of lesions that have not been detected by white-light examination was not the only target of our study. Because the patients in our series were admitted to our department to rule out invasive cancer, sensitivity and specificity from our study have to be interpreted with caution and cannot be readily applied to the general population. To evaluate the VELscope as screening tool in oral cancer and precancer detection, further prospective trials with adequate follow-up and histologic confirmation have to be conducted in a primary care setting. However, regarding the results of a cluster-randomized controlled trial in a high-risk population in India, it seems to be appropriate to focus on male users of tobacco and alcohol.48 Up to now, no prospective trial has confirmed that occult lesions were identified with the VELscope that have not been diagnosed by conventional white-light examination and palpation alone. It should be noted that a subset of oral carcinomas arise from clinically normal-appearing mucosa.7,49 Besides the use as an adjunctive tool in identifying suspicious lesions, several authors highlighted the value of the VELscope for identification of the most appropriate region for biopsy. In superficial nonhomogeneous lesions, the loss of autofluorescence can lead to the region most suitable for an incisional biopsy.34,39 Additionally, fluorescence loss associated with oral cancer detected with VELscope has been used to identify tumor margins before resection.46

Regarding the characteristics of a good screening test as proposed by Lingen et al.,27 it can be stated that
autofluorescence assessment of mucosa lesions with the VELscope is simple, safe, and acceptable for the patients. However, interpretations of the results as well as documentation are crucial and require appropriate training and knowledge. Although some studies showed promising results in locating malignant and premalignant lesions, the discriminatory power between malignant and other conditions remains questionable because of the low positive predictive value.

CONCLUSIONS

Autofluorescence imaging is an easy and fast approach for obtaining additional information of suspicious mucosa lesions. As shown previously by other groups, malignant and dysplastic lesions present as areas with fluorescence loss. Although all carcinoma and dysplastic lesions in our series were identified with the VELscope, autofluorescence imaging results have to be interpreted with caution, because benign reactive lesions may show similar auto-fluorescence patterns, just as dysplasia or invasive carcinoma. Additional histology controlled prospective studies in a general population are required to assess the role of auto-fluorescence imaging as a screening adjunct.

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Appendix O
Narrow band (light) imaging of oral mucosa in routine dental patients. Part I: Assessment of value in detection of mucosal changes

Edmond L. Truelove, DDS, MSD • David Dean, DDS • Samuel Maltby • Matthew Griffith
Kimberly Huggins, RDH • Mickealla Griffith, DDS • Stuart Taylor, DDS, MSD

The purpose of this investigation was to determine the value of adding narrow band (light) imaging (NBI) to the standard oral soft tissue examination process used to detect mucosal change. A total of 620 dental patients who came to the clinic for regular dental evaluation or for treatment of acute dental problems were given a standard oral soft tissue examination by dental students under faculty supervision. The results of the white light examination were recorded after the tissues were examined with NBI, at which point areas with a loss of fluorescence (LOF) were recorded. The nature of the tissue change was classified clinically as normal variation, inflammatory, traumatic, dysplastic, or other, and patients were categorized depending on their clinical findings: normal, need follow-up visit, or immediate biopsy. Risk factors related to oral dysplasia also were recorded. The addition of NBI added between one and two minutes to the examination process.

Of the 620 examinations, an area with an LOF suggestive of pathology was detected in 69 subjects (11.1%). After a second immediate evaluation, 28 of the 69 subjects were scheduled for follow-up or biopsy. None of the lesions discovered in these 28 subjects had been detected using standard (white light) examination.

Adding NBI to the routine clinical examination resulted in detection of changes not seen with white light examination in 11.1% of patients; of these, a small but important number were found to have otherwise undetected persistent changes representing inflammatory lesions or potentially dangerous oral dysplasia. Adding NBI as an adjunctive diagnostic procedure improved the quality and outcome of the examination process.

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A n important component of dental practice is the detection of changes to the oral mucosa and jaws that represent serious threats to health. Among these threats, the risk of oral cancer is a chief concern. Although the overall risk for cancer of the mouth and throat is relatively small, data from the American Cancer Society and National Cancer Institute predict that the lifetime risk of oral cancer is 1 in every 152 females and 1 in every 71 males.1 The lifetime risk for developing oral cancer is greater than the lifetime risk for cancers of the brain, esophagus, and lymphomas, conditions that receive frequent public scrutiny as important risks for reduction in life expectancy.1 Oral cancer also is a significant problem because survival rates have improved only marginally during the past 50 years, with the five-year survival rate still only 53%.1

Important risk factors for oral cancer include age, ethnic status, tobacco use, excess alcohol consumption, family history of cancer, and prior cancers.2 The presence of some types of mucosal change, including leukoplakia, erythroplakia, proliferative verrucous leukoplakia, and lichen planus, also has been associated with an increased risk.3,6 Poor oral hygiene and lack of regular dental care are among suggestions as potential risk factors, either because of local inflammatory irritation or because patients with poor access to care do not benefit from earlier detection of mucosal changes.7 Chronic mucosal infections, including candidiasis, herpes simplex, and human papilloma virus, also have been postulated as causing an increased risk for oral cancer.8-10

A factor that could be associated with poor prognosis is a delay in the detection and treatment of early oral cancers; however, data to support that hypothesis are not extensive.1,11-13 Still, if oral cancer behaves like most other cancers, it is logical to assume that very early detection and treatment is likely to result in better survival than delayed detection, which usually is associated with wider spread, metastatic nodes.
and regional spread to other organs. Some data exist that identify rates of progression from benign and premalignant to malignant for several types of oral lesions, but little actual data have been collected to demonstrate the value of routine oral examination of patients on reducing the risk of cancer and cancer morbidity.11,12 Some authors have suggested that there is little significant information to support the use of routine oral examination as a valuable tool to reduce morbidity or mortality.13

One of the difficulties associated with the clinical assessment of patients who could be at risk for oral cancer is that, until very recently, the only diagnostic method available has been visual and tactile examination of the oral mucosa. While that diagnostic process is reasonable, it cannot detect cellular changes that have not evolved enough to be visible to the unaided eye.

In the past, cancer detection and surveillance in other organ systems have suffered from the same limitations, with purely clinical observations proving to be inadequate in detecting premalignant or early malignant changes. Two excellent examples include the poor predictive value of visual inspection of the uterine cervix and breast self-examination. Until initiation of colposcopy and Pap smear evaluation of the cervix, cancer rates and deaths were significantly higher, while mammography has greatly improved detection and survival of patients with breast cancer.14,15 All three techniques are considered adjunctive diagnostic procedures designed to provide data to the clinician which, when included in a symptom report and risk factor assessment, can lead to more effective decision-making about the likelihood that a finding represents a potential neoplastic process that requires a biopsy or other more sophisticated diagnostic procedures.

The lack of effective adjunctive clinical diagnostic methods has clearly limited the ability of dental professionals to detect very early changes that could predict the presence of emerging inflammatory, premalignant, and dysplastic changes, leaving only visual inspection as the chief diagnostic tool. After visual detection of an observable change in the mucosa, clinicians have had access to two adjunctive diagnostic tools and one definitive tool to guide their decision-making: cytology, toluidine blue tissue staining, and biopsy.16,17 These methods have helped clinicians to decide whether a finding deserves more careful follow-up and management, and while all three methods remain important and valuable, they still are limited due to their dependence on the presence of visible tissue changes to alert the clinician that further assessment is needed.

Methods to improve early detection of mucosal changes prior to their progression to a frank, clinical lesion state could improve prognosis and limit the morbidity associated with treatment. Narrow band (light) imaging (NBI) of tissues has been used extensively in other areas of the body as a means of identifying tissue changes that are either not visible to the unaided eye or uncharacteristic of a neoplastic process.18-20 This method has been used to evaluate bronchial tissues and the mucosa of the intestinal tract, with findings that have demonstrated its potential utility.18-20 Recently, studies funded by the NIH have investigated the use of NBI for the detection of changes.
in the oral mucosa associated with neoplasia or premalignant cellular change.\textsuperscript{21,22} These studies have shown that NBI has value in the detection of malignant disease and in the determination of surgical margins.\textsuperscript{23} One result of these studies has been the development, FDA approval, and marketing of a NBI instrument, VELscope (LED Dental Inc.), that is designed for use in general practice settings.\textsuperscript{24} Similar instruments are currently under development.

NBI uses a blue light directed at the oral mucosa and observed through an eyepiece that filters the light. Tissues with different physical, vascular, and cellular characteristic reflect or absorb the blue light, resulting in an image as viewed through the scope with different visual characteristics. The blue light augments the fluorescence properties of some tissue components, generating a green-white appearance. On the other hand, the optical characteristics of some tissues result in a loss of fluorescence (LOF), causing a dark pattern when the tissues are observed through the scope. Inflamed and highly vascularized tissues absorb the light and appear dark compared to the same tissue without inflammation. Oral dysplasia and oral cancer also absorb the light and appear darker than the corresponding tissue without cancer or dysplasia. Dysplastic tissues with significant keratinization (leukoplakia) can exhibit increased fluorescence (whiteness) with LOF (darkness) around the periphery of the lesion. Obviously, because inflammatory lesions absorb the light and appear dark, traumatic, viral, and aphthous lesions demonstrate an LOF, as do migratory glossitis and lymphoid tissue (Fig. 1–8).

Critics of the use of NBI have argued that the results are not sensitive or specific enough and can result in “false positive” findings that cause patients to be at risk for unnecessary invasive procedures.\textsuperscript{24,25} Others argue that the use of such adjunctive diagnostic devices is not necessary because risky mucosal changes are visible and can be detected with the unaided eye.\textsuperscript{26}

The difficulty with those opinions is that very early changes at the cellular level occur before the gross physical characteristics of the tissue have changed enough to create a clearly visible lesion that, when seen by the clinician, registers as a potentially important inflammatory or dysplastic lesion. Also, most adjunctive diagnostic methods are merely that—adjunctive—and are not intended to be definitive diagnostic tests. Application of strict standards of sensitivity and specificity in judging the relative value of these adjunctive methods could underestimate their potential for guiding the initial clinical decision-making as part of an overall assessment algorithm. Their chief use is to help clinicians discover changes that otherwise might not be observed or be of such a subtle nature that the clinician disregards the potential significance of the finding.

One study that assessed the value of NBI and toluidine blue in determining the nature of clinically detected lesions in a large group of adults who received oral examinations concluded that use did not improve the diagnosis of oral cancer; however, NBI was applied to only those patients who had clearly detectable oral lesions rather than being used as an adjunctive diagnostic process for all of
the examinations. Had this been done, it is likely that more cases of early dysplasia would have been detected. Application of the technology on all patients could have helped the examiners to identify changes that otherwise would have escaped recognition because of their nonspecific characteristics or lack of progression to a clearly visible state. Unfortunately, only a few studies have evaluated the application of NBI in routine dental practice, but one study has shown detection of premalignant changes that otherwise would have escaped detection.

**Objectives**

The purpose of this study was to evaluate the value of adding NBI of the oral mucosa for the detection of tissue changes to a standard oral examination in routine dental patients. The study also aimed to assess the relative value of NBI in the detection of inflammatory, dysplastic, and other tissue changes. The goal of the study was to assess the value of adding NBI for the detection of oral changes not readily seen during normal, white-light examination of the oral mucosa. The purpose of the study was not to determine the absolute value of NBI in the detection of oral dysplasia or oral cancer, but to assess whether its use as an adjunctive diagnostic method adds value to standard examination processes. The study also was designed to test the value of this adjunctive method after only a brief examination to determine its value in normal general practice settings, rather than in settings where the modality would be employed by experts who regularly engage in diagnosis and management of mucosal lesions.

**Materials and methods**

**Subjects**

Patients seeking routine dental care or treatment for dental symptoms (pain, toothache, and so forth) were invited to participate in the study protocol. The study was approved as a quality improvement study by the institutional review board of the University of Washington, and all patients entered into the study and signed consent after being informed of the study by one of the study investigators.

**Study protocol**

The study protocol included the following elements: Introduction of the patient to the study and obtaining consent to participate; routine social, medical, and dental histories; a head and neck physical examination, oral soft tissue assessment, and dental examination; recording of visual findings using a data collection form, scoring of tissue changes, and level of dysplasia suspicion (0–4); examination of mucosal tissues using a narrow band light source (VELscope), followed by recording the findings; scoring of type of tissue change and level of dysplasia suspicion (again, on a 0–4 scale); recording follow-up designations as None, Two-week, Four-week, Biopsy Next Visit, Biopsy This Visit, and Other; and recording of risk factors, including none, tobacco, alcohol, immunosuppressive disorder, immunosuppressive medication, cancer history, diabetes, and family history of cancer.

All patients were examined initially by third- and fourth-year dental students, then by the attending faculty of the clinic. Students were provided with a tutorial on conduct of the clinical and NBI methods with examples of normal findings, normal variation, changes caused by inflammatory disorders, and changes caused by dysplasia. The faculty of the clinic was provided with the same information as the students in a computer-based tutorial format. In addition, students and faculty were provided with an instruction packet for each patient enrolled in the study that...
Five percent of subjects declined participation in the study after reading the consent form and discussing the study with an investigator. The most typical reason for a patient declining was concern that the light would cause harm or fear that an abnormality would be detected. Overall, patients were very accepting of the procedure and expressed great appreciation that an adjunctive noninvasive diagnostic aid was available for their evaluation. The addition of the NBI protocol to the examination process added one to two minutes to the visit, not including the study consent process that is not part of a routine diagnostic procedure. Many patients reported personal experiences with friends or relatives who had developed oral cancer and other diseases of the mouth and commented positively about the thorough process being employed at the clinic.

Patients ranged in age from 18–85, and 55% of the 620 patients were women. Of the patients who reported tobacco use, 21.5% reported active use and 15.5% reported prior tobacco use, with only a few patients reporting the use of smokeless tobacco. Nine percent of patients reported a prior history of some type of cancer, and 57% reported a family history of cancer. Nine percent of patients were diabetic and currently under treatment, while 7.5% identified themselves as having an immunological disorder or having used an immunosuppressive medication (Table 1).

LOF in areas that were reported as normal during the white light examination was detected in 69 patients. After immediate re-evaluation, 41 patients were determined to have a region of subtle LOF that could be explained by normal variations in tissue characteristics, while 28 patients were scheduled for either immediate biopsy or a follow-up appointment. Five of those patients agreed to an immediate biopsy and four decided to follow up with their primary dental provider. The remaining 19 patients were scheduled for follow-up in two weeks. Of the 15 patients who returned for reassessment, the area of LOF had resolved and no clinical or NBI abnormality could be detected for 11 of them; this left four patients with persistent LOF compared to corresponding tissues. These LOF sites were biopsied in the same manner as the sites in the five patients who agreed to an immediate biopsy.

In all, nine patients (five during the initial assessment and four at the follow-up visit) were found to have tissue changes detected with NBI, but not white light, that were significant enough when considered in conjunction with the patient’s history to require further diagnostic assessment. After the findings and risks were explained in addition to the alternatives to biopsy, all nine patients consented to biopsy.

Table 1. Oral cancer risk factors for patients in this study (n = 620).

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Percentage of all patients enrolled</th>
<th>Percentage of patients with significant LOF (n = 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current tobacco use</td>
<td>21.5</td>
<td>32.1</td>
</tr>
<tr>
<td>Prior tobacco use</td>
<td>15.5</td>
<td>21.0</td>
</tr>
<tr>
<td>History of excess alcohol use</td>
<td>3.5</td>
<td>5.0</td>
</tr>
<tr>
<td>Poor oral hygiene</td>
<td>14.5</td>
<td>15.6</td>
</tr>
<tr>
<td>Diabetic in active treatment</td>
<td>9.5</td>
<td>11.5</td>
</tr>
<tr>
<td>History of any type of cancer</td>
<td>9.0</td>
<td>12.5</td>
</tr>
<tr>
<td>History of autoimmune disease or immunosuppressive medication</td>
<td>7.5</td>
<td>14.2</td>
</tr>
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</table>
although two of them received the biopsy at another facility due to insurance issues.

Of the nine patients who underwent biopsy, three were classified by histopathological assessment as having mild dysplasia and two were classified as having mild to moderate dysplasia (Chart 1). Two other patients were diagnosed as being histologically compatible with lichen planus, and the remaining two patients had inflammatory lesions (Table 2). Lesions detected during the white light examination were not included in this discussion and were handled in the routine manner used to manage visible oral lesions. The five dysplastic lesions that were detected with NBI were located in the buccal mucosa, the lateral border of the tongue, the lip, the palate, and the alveolar ridge.

The white light examination resulted in the detection of a variety of soft tissue lesions of the mucosa, but this study did not focus on those that were easily detected using standard visual inspection techniques. For the sake of completeness, a brief summary of the types of soft tissue lesions encountered using white light and NBI is listed in Table 3. These lesions included cheek bites, aphthous ulcers, herpetic lesions, migratory glossitis, fissured tongue, lichen planus, inflamed minor salivary duct openings, candidiasis, and cheilitis. Tonsillitis, pharyngitis, papillomas, scars, leukoplaikia, and draining abscesses also were detected. Those lesions with inflammatory components demonstrated LOF, and in most cases the LOF provided a more dramatic presentation of the extent and severity of the inflammatory change than the clinical examination did (Fig. 5–8).

The mucosal changes detected with white light, both white light and NBI, or NBI only were widely distributed throughout the mouth, with no distinct difference in pattern noted between the two different methods of assessment.

As previously described, a number of patients had mucosal changes detected with one or both types of visual assessments. Changes were noted in nearly half of all patients (305 of 620); however, the vast majority of them were found to be normal or minor variants and did not appear to represent significant pathology. The most common lesion was cheek bite, while the second most common was trauma to the tongue. Inflammatory changes to the oropharyngeal and tonsil areas also were common. Cheilitis and changes to the epithelium of the lips also were common and represented a range of etiologies that included habitual lip biting and actinic changes of the lower lip. A number of cases of lichen planus and generalized glossitis also were detected during the white light examination.

Although the study size was reasonably large, the diverse nature of lesions found and the wide range of risk factors associated with the development of oral lesions precluded development of specific associations between risk of mucosal change and a host of factors, including age, gender, tobacco use, diabetes, immunodeficiency, immunosuppressive medications, cancer history, family cancer history, and oral health status. Nevertheless, it is interesting to note that the patients with changes detected with white light, NBI, or both were more likely to carry one or more of the risk factors compared to those who had no areas of mucosal

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**Chart 1. Flow diagram of the study results.**

<table>
<thead>
<tr>
<th>Event</th>
<th>Number</th>
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<tbody>
<tr>
<td>Patients seeking routine dental evaluation and urgent care (n = 652)</td>
<td></td>
</tr>
<tr>
<td>Refused enrollment (n = 32)</td>
<td></td>
</tr>
<tr>
<td>Enrolled (n = 620)</td>
<td></td>
</tr>
<tr>
<td>Loss of fluorescence (n = 69)</td>
<td></td>
</tr>
<tr>
<td>Area judged as low risk or normal variant (n = 41)</td>
<td></td>
</tr>
<tr>
<td>Follow-up visit (n = 19)</td>
<td></td>
</tr>
<tr>
<td>Area judged to require further evaluation (n = 28)</td>
<td></td>
</tr>
<tr>
<td>Lost to follow-up (n = 4)</td>
<td></td>
</tr>
<tr>
<td>Returned for evaluation (n = 19)</td>
<td></td>
</tr>
<tr>
<td>Area resolved (n = 15)</td>
<td></td>
</tr>
<tr>
<td>Biopsied at follow-up (n = 4)</td>
<td></td>
</tr>
<tr>
<td>Immediate biopsy (n = 5)</td>
<td></td>
</tr>
<tr>
<td>Dysplasia (n = 5)</td>
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</table>
change, with 54 of 69 patients (78%) who demonstrated LOF having either a history of tobacco use or current tobacco use. Those with mucosal lesions also were more likely to have poor oral hygiene.

Discussion
The purpose of this quality improvement study was to gain information about the clinical utility of one simple adjunctive diagnostic method (NBI) for the detection of mucosal changes. The rationale for the study assumed that such a diagnostic adjunctive method is not necessary to detect mucosal changes readily seen with normal white light examination methods. Existing data suggest that current examination methods are not sufficient for the earliest detection of mucosal changes that could represent inflammatory damage or the presence of very early dysplasia. This could partly account for the only modest reduction in oral cancer deaths since 1960.1,13

There are several possible explanations for why oral cancer deaths and the stage of oral cancer at the time of diagnosis have not changed dramatically in the past 50 years.1 The lack of improvement could relate to a number of factors, but when considering that the percentage of the population that receives regular dental care has increased in the past 50 years, it appears obvious that current diagnostic methods could benefit from one or more adjunctive approaches. Early detection of dysplasia in other organ systems has been acknowledged to be an important component in improving survival, so it is difficult to believe that early detection of potentially significant mucosal changes, whether they are inflammatory or dysplastic, would not lead to improvements in cancer-related outcomes.

Because oral cancer is a relatively uncommon condition, the authors did not expect to detect a large number of cases of dysplasia with either the white light examination or the use of NBI and were surprised that five cases of early dysplasia were identified. Of additional interest is the observation that NBI detected many areas of inflammation and vascular change not identified during the white light examination, suggesting that this methodology also could be useful in cataloguing instances of chronic irritation and inflammatory change that, over time, could lead to irreversible conditions such as fibrosis, scarring, and leukoplakia.

Table 2. Biopsy results.

<table>
<thead>
<tr>
<th>Lesion diagnosis</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lichen planus</td>
<td>2</td>
</tr>
<tr>
<td>Inflammation</td>
<td>2</td>
</tr>
<tr>
<td>Mild dysplasia</td>
<td>3</td>
</tr>
<tr>
<td>Mild to moderate dysplasia</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 3. Types of lesions detected with combined clinical and NBI diagnosis methods.

<table>
<thead>
<tr>
<th>Type of mucosal lesion detected</th>
<th>Relative frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Traumatic injury</td>
<td>Common</td>
</tr>
<tr>
<td>Lichen planus</td>
<td>Occasional</td>
</tr>
<tr>
<td>Dysplasia</td>
<td>Rare*</td>
</tr>
<tr>
<td>Cheilitis</td>
<td>Common</td>
</tr>
<tr>
<td>Migratory glossitis</td>
<td>Occasional</td>
</tr>
<tr>
<td>Fissured tongue</td>
<td>Occasional</td>
</tr>
<tr>
<td>Pharyngitis and tonsillitis</td>
<td>Common</td>
</tr>
<tr>
<td>Herpes simplex</td>
<td>Occasional</td>
</tr>
<tr>
<td>Recurrent aphthous</td>
<td>Occasional</td>
</tr>
<tr>
<td>Candidiasis</td>
<td>Occasional</td>
</tr>
<tr>
<td>Leukoplakia</td>
<td>Occasional</td>
</tr>
<tr>
<td>Mucosal bacterial infections</td>
<td>Rare</td>
</tr>
<tr>
<td>Inflamed minor salivary ducts</td>
<td>Occasional</td>
</tr>
</tbody>
</table>

Common = ≥10% or greater; occasional = <10%; rare = <1%.

*Near 1% prevalence in this study’s population.
routine dental patients seen in private practice settings because more than 60% of the patients enrolled in the study were seeking urgent care and might have had more risk factors (tobacco, poor oral hygiene, systemic disease, and so forth) than normal dental populations.

The study methodology was limited because it was carried out in a clinical setting that did not allow for a reduced ambient light examination environment. Based on the authors’ experience in the use of NBI in darker settings, it is likely that a number of lesions viewed at the clinic with LOF went undetected. It is possible that one or more of these lesions might even have been dysplastic or an inflammatory change that could have benefited from further follow-up.

The study also was limited because the authors deliberately decided to use relatively inexperienced examiners, which might have resulted in lower rates of detection of mucosal changes for either method. The authors wanted to test the use of NBI in an environment that resembled a general dental setting more than a specialty clinic that focuses on the detection of mucosal lesions and disease. To that end, the results demonstrate the value of NBI when added to routine examination methods.

The study also could have been limited because it occurred in a university setting, where students and attending faculty might be more focused on mucosal assessment processes. A larger, multiple private office study would be useful, with general dentists and dental hygienists providing the white light and NBI process during normal patient care for both new and recall patients. It is encouraging, however, that this adjunctive diagnostic aid appeared to improve the detection of mucosal changes not easily visible with white light examination.

The authors were pleased that adding the NBI to the examination process did not significantly increase the time required to evaluate patients when the study consent process was excluded. The authors also were pleased that patient response was strongly positive and that the study appeared to raise awareness among patients that the dental examination process extends beyond purely odontogenic issues and can encompass the detection of disorders that could have more severe and wider implications on their health.

Conclusion
The findings of this study support the use of NBI as a simple adjunctive diagnostic device that, when used as one component of a standard diagnostic protocol, could help clinicians to detect inflammatory and dysplastic tissues. Use of this technology could improve clinicians’ ability to monitor and follow initially detected changes, and to better judge progression versus resolution and response to nonsurgical treatments. These findings need to be further explored in other settings to determine overall utility in general practice, but based on these findings, NBI appears to have the potential to assist general practitioners in assessment and decision-making related to mucosal tissues and lesions.

Acknowledgements
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References
Exercise No. 287

Cancer Screening

Subject Code 736
The 15 questions for this exercise are based on the article “Narrow band (light) imaging of oral mucosa in routine dental patients. Part I: Assessment of value in detection of mucosal changes” on pages 281-289. This exercise was developed by Daniel S. Geare, DMD, in association with the General Dentistry Self-Instruction committee.

Reading the article and successfully completing the exercise will enable you to:
- understand the types of mucosal abnormalities;
- understand clinical methods of diagnosing mucosal abnormalities; and
- understand the value of narrow band (light) imaging in identifying mucosal changes.

1. Narrow band (light) imaging (NBI) is effective in detecting
   A. dysplastic tissue changes.
   B. changes in tissue color.
   C. tissue thickness.
   D. discrepancies between the tooth and the periodontium.

2. The purpose of NBI includes all of the following except:
   A. Establishing the value of NBI in older, at-risk patients
   B. Studying the value of adding NBI to a standard oral examination
   C. To establish whether NBI can detect oral cancer
   D. To determine whether oral examinations can be improved using NBI

3. The lifetime risk for developing oral cancer is greater than the risk of developing all of the following cancers except:
   A. Brain
   B. Esophageal
   C. Lymphatic
   D. Pancreatic

4. What is the five-year survival rate for oral cancer?
   A. 25%
   B. 46%
   C. 53%
   D. 69%

5. Which of the following has been associated with an increased risk of oral cancer?
   A. Erythroplakia
   B. Chronic bacterial infection
   C. Chronic periodontitis
   D. Acute traumatic injuries

6. Why is a visual and tactile examination limited in detecting oral cancer?
   A. It cannot detect cellular changes
   B. It is limited by the texture of the lesion
   C. There are too many false positives
   D. Oral lesions are too variable in color

7. Which of the following is not a risk factor for oral cancer?
   A. Ethnic status
   B. Excess alcohol consumption
   C. Prior cancers
   D. Chronic periodontal disease

8. NBI has been shown to have value in detecting
   A. the extent of decay.
   B. malignant disease.
   C. blood flow in inflamed tissues.
   D. the quality of the attached gingiva.

9. Diagnostic tools currently available to clinicians to measure mucosal changes include all of the following except:
   A. Staining
   B. Cytology
   C. Biopsy
   D. Computer imaging

10. Methods used to improve early detection of mucosal changes can
    A. improve prognosis.
    B. extend morbidity.
    C. control mortality.
    D. lower incidence.
11. NBI uses a blue light that detects all of the following tissue changes except:
   A. Physical
   B. Vascular
   C. Cellular
   D. Texture

12. Inflamed and highly vascularized tissues absorb the light and appear dark. Oral cancer, by contrast, also absorbs light but appears lighter due to the cellular changes of the cancer cells.
   A. Both statements are true.
   B. The first statement is true; the second is false.
   C. The first statement is false; the second is true.
   D. Both statements are false.

13. During this study, dysplastic lesions were detected in all of the following sites except:
   A. The lateral border of the tongue
   B. The lip
   C. The cheek
   D. The alveolar ridge

14. Which of the following was among the most common mucosal changes detected by the white light examination?
   A. Cheek biting
   B. Tongue bites
   C. Candidiasis
   D. Bacterial infection

15. There has been only a modest reduction in oral cancer deaths in the past 40 years. The existing data indicate that the examination methods have been insufficient for the early detection of mucosal changes.
   A. Both statements are true.
   B. The first statement is true; the second is false.
   C. The first statement is false; the second is true.
   D. Both statements are false.

Answer form and Instructions are on pages 319-320.
Answers for this exercise must be received by June 30, 2012.
EFFICACY OF TISSUE AUTOFLUORESCENCE IMAGING (VELSCOPE) IN THE VISUALIZATION OF ORAL MUCOSAL LESIONS

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Abstract: Background. Technology that highlights potentially malignant oral lesions in a highly sensitive and specific manner will aid clinicians in early diagnosis of these conditions. This study assessed the efficacy of direct tissue autofluorescence imaging Visually Enhanced Lesion Scope (VELScope) in the detection of oral mucosal lesions.

Methods. One hundred twelve patients referred with a potentially malignant oral mucosal lesion were examined under routine incandescent light, and then with VELScope, noting loss of autofluorescence and presence of blanching. Incisional biopsies were performed to provide definitive histopathological diagnoses.

Results. VELScope enhanced the visibility of 41 lesions and helped uncover 5 clinically undetected lesions. VELScope examination alone showed a sensitivity of 30% and a specificity of 63%. Its accuracy at identifying dysplasia was 55%.

Conclusion. VELScope examination cannot provide a definitive diagnosis regarding the presence of epithelial dysplasia. Loss of autofluorescence is not useful in diagnosing epithelial dysplasia in its own right without relevant clinical interpretation.

Keywords: oral cancer; early detection; oral examination; tissue autofluorescence imaging; VELScope

Oral squamous cell carcinoma (OSCC) ranks as the sixth most common malignancy worldwide, with approximately 400,000 new cases diagnosed in the year 2007.1 The overall survival rate for OSCC is approximately 50%,2,3 despite technological advancements in diagnostic and treatment procedures. High morbidity and mortality rates call for early detection and treatment of precancerous and cancerous lesions,1–10 but this is dependent on visualization of lesions and stratification of benign from potentially malignant lesions. Traditionally, conventional oral examination for oral cancer involving visual inspection and digital palpation of oral lesions under projected incandescent or halogen illumination has been deficient.6,11–13 Conventional oral examination is a poor differentiator for benign, dysplastic, and malignant lesions, thereby resulting in delayed patient referral and poorer prognosis.6,7,11 Conventional oral examination is unable to accurately discriminate between progressive and nonprogressive lesions; neither does it accurately detect precancerous lesions which present in clinically normal mucosa.13 These findings have driven the development of new technologies that aim at highlighting potentially malignant oral lesions in a highly sensitive and specific manner to aid clinicians in early diagnosis and treatment of these conditions.14–17

Visually Enhanced Lesion Scope (VELScope) is a simple, noninvasive, handheld device that permits direct visualization of alterations to tissue fluorescence and is marketed as a screening device for oral cancer.13,14 VELScope produces a blue excitation light in the wavelength range of 400 to 460 nm which aims to excite green fluorescence from endogenous fluorophores in oral tissue.14 Optical changes are said to reveal metabolic, biochemical, and structural information about the mucosal cells and map the field of can- cerization.18 Normal oral mucosa emits a pale green autofluorescence and is said to retain fluorescence while abnormal tissue shows loss of autofluorescence and appears dark in contrast.15,19

The manufacturer of VELScope (LED Dental, Vancouver, British Columbia, Canada) states that it is intended for use by a dentist or health-care provider as an adjunct to clinical oral examination to enhance the visualization of oral mucosal abnormalities that may be or may lead to oral cancer and that may not be apparent or visible to the naked eye. The device is also intended to be used by a surgeon to help identify diseased tissue around a clinically
apparent lesion and thus aid in determining the appropriate margin for surgical excision.

Tissue fluorescence imaging is said to be superior to clinical judgment when determining size, extent, and distribution of cancer field as the optical changes expose subclinical genetic alterations. Numerous studies have used fluorescence imaging in the detection of malignant and potentially malignant oral lesions, however, those that have used VELScope involved patients who were known to have a history of dysplasia or carcinoma in situ, and as such, the case study design of these studies does not hold much evidential weight when it comes to changing clinical practice.

The main purpose of the current study, therefore, was to evaluate VELScope as a diagnostic aid for oral cancer and precancer detection in a prospective screening manner conducted by oral medicine specialists. A secondary purpose was to assess the effect of diascopic fluorescence.

**MATERIALS AND METHODS**

Patients presenting to an oral medicine specialist unit for assessment of an oral mucosal lesion were recruited into the study. One hundred twelve patients were enrolled over a 6-month period. The only criterion for inclusion in the study was referral for examination of an oral mucosal white or mixed red/white lesion that was deemed by the referring general practitioner to be clinically suspicious and warranted further evaluation by an oral medicine specialist. Patients known to have oral epithelial dysplasia or squamous cell carcinoma were not included in this study. The study was conducted according to Human Ethics Guidelines approved by the Universities of Queensland and Melbourne. Participation in the study was voluntary and followed informed consent. The age, sex, smoking history, and alcohol consumption habits of all patients were recorded. Conventional oral examination was performed under incandescent operatory light, and a provisional clinical diagnosis was recorded. The location, size, ease of visibility, and border distinctness were recorded as previously described. Lesions were grouped into 4 categories: (1) homogenous leukoplakia/keratosis (ie, a nonwipeable homogenous white patch with no apparent etiology), (2) nonhomogenous leukoplakia or clinically suspicious for malignancy (ie, mixed red-white or mixed red-white ulcerated lesions with a high index of suspicion for dysplasia or squamous cell carcinoma), (3) lesions with lichenoid features suggestive of oral lichen planus/oral lichenoid reaction (ie, a characteristic distribution of white reticular lesions with or without erosion or ulceration), and (4) others.

Clinical examination was repeated using VELScope while the room and operatory lights were dimmed, and all measurements repeated. Lesions that showed loss of autofluorescence were deemed positive, and lesions that did not show any loss of autofluorescence were deemed negative. In addition, all lesions that lost autofluorescence were blanched to evaluate diascopic fluorescence, and those that were deemed negative for loss of autofluorescence only if complete blanching was achieved. Following VELScope examination, a provisional VELScope diagnosis was also recorded for each lesion. All lesions were photographed (Figure 1) as previously described for future review and correlation, and these were not used for the initial clinical diagnoses. Calibration of clinical observations was also undertaken between 2 of the authors (C.S.F. and M.J.M.) on a separate cohort of patients not included in this study.

A scalpel tissue biopsy was taken with the patient under local anesthesia for all lesions to obtain a definitive histopathological diagnosis which would serve as the gold standard for future comparisons. Routine features of nonhomogeneity were used to determine the biopsy site under incandescent light. Loss of
autofluorescence was used to determine the best site for biopsy with VELScope. Areas that showed both nonhomogeneity and loss of autofluorescence were chosen in preference to areas that showed only one or the other feature. Biopsy specimens were fixed in formalin, blocked in paraffin, stained with hematoxylin–eosin, and assessed by routine histopathology by an experienced pathologist not involved with the clinical arm of the study, in addition to 1 of the authors (C.S.F.). Both examiners were blinded to the clinical findings and used recognized measures of interpretation for oral epithelial dysplasia.26

Sensitivity, specificity, accuracy, and positive and negative predictive values were calculated for the provisional diagnoses using conventional oral examination alone, VELScope findings alone, and after consideration of both the conventional oral examination and VELScope in combination, these were compared to the histopathological diagnosis as the gold standard. Receiver operator characteristic (ROC) space analysis was also undertaken.

Quantitative data were analyzed using the statistical features of GraphPad Prism Version 4.01 (GraphPad, San Diego, CA). A histopathological diagnosis of dysplasia was considered positive for the presence of disease in all examinations and comparisons (conventional oral examination, VELScope oral examination, and the combined examination), and no distinction was made between mild, moderate, or severe dysplasia in regards to this comparison. In addition to quantitative data, subjective qualitative analysis was undertaken by recording answers to the following questions: (1) Did VELScope examination enhance visualization?; (2) Did VELScope examination enhance lesion visibility and border distinctness?; (3) Did VELScope examination uncover a new lesion?; (4) Did VELScope examination alter the provisional clinical diagnosis?; and (5) Did VELScope examination alter the chosen biopsy site?

RESULTS
A total of 112 patients were examined with VELScope: 46 men and 66 women (Table 1). Fifty-four patients were current users of tobacco (26.9 ± 2.67 pack years), 47 were regular alcohol consumers, and of these, 31 were both tobacco and alcohol consumers. Mean lesion area did not significantly change with

### Table 1. Demographic information of patients examined (total N = 112).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Male (n = 46)</th>
<th>Female (n = 66)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean ± SD, y</td>
<td>57.8 ± 11.88</td>
<td>59.08 ± 12.8</td>
</tr>
<tr>
<td>Smoker, n</td>
<td>22</td>
<td>32</td>
</tr>
<tr>
<td>Alcohol consumer, n</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>Smoker &amp; alcohol, n</td>
<td>15</td>
<td>16</td>
</tr>
</tbody>
</table>

### Table 2. Visual characteristics of oral lesions (primary and satellite) examined with and without VELScope visualization (n = 161).

<table>
<thead>
<tr>
<th>Lesion features</th>
<th>Visual inspection</th>
<th>VELScope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visability score, mean ± SEM</td>
<td>3.34 ± 0.06</td>
<td>3.22 ± 0.09</td>
</tr>
<tr>
<td>Distinct border, %</td>
<td>56</td>
<td>61</td>
</tr>
<tr>
<td>Lesion area, mean ± SEM, mm²</td>
<td>109.1 ± 16.93</td>
<td>116.6 ± 18.49</td>
</tr>
</tbody>
</table>


No significant difference was detected for visibility or lesion area.

VELScope examination, although on average there was a slight increase in lesion size when viewed with VELScope (Table 2).

Of the 112 patients examined, all underwent biopsies resulting in 118 specimens, with the majority of lesions occurring on the buccal mucosa and tongue (Table 3). Provisional clinical and correlating VELScope diagnoses, as well as the definitive histopathological diagnoses, are listed in Table 4. Tissue autofluorescence characteristics of biopsied lesions are shown in Figure 2. The results shown in Figure 2 are further analyzed for accuracy in Tables 4 and 5, and these datasets should be interpreted together.

There was a 96.6% interobserver agreement for histopathological interpretation between the 2 pathologists, with differences mainly limited to the grade of dysplasia. When there was a discrepancy, the more severe grade was always used in the final diagnosis.

Correlation of clinical oral examination, VELScope findings, and provisional diagnosis after consideration of both the conventional oral examination and VELScope compared with histopathological findings was undertaken (Table 5). ROC space analysis is shown in Figure 3.

Clinical interobserver agreement was calculated with 71.4% agreement on the clinical provisional diagnosis, 60.7% agreement on the VELScope provisional diagnosis, 82% agreement on loss of autofluorescence, and 62% agreement on complete blanching after loss of autofluorescence. Once again, when a discrepancy was noted, the more severe diagnosis and loss of autofluorescence finding was used in the final dataset.

The results of the 5 qualitative questions posed in the Methods section were as follows: VELScope examination enhanced lesion visualization of 41 lesions, and uncovered 5 clinically undetected lesions (1 of

### Table 3. Location of biopsied lesions (n = 118).

<table>
<thead>
<tr>
<th>Location</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tongue</td>
<td>29</td>
</tr>
<tr>
<td>Buccal mucosa</td>
<td>40</td>
</tr>
<tr>
<td>Floor of mouth</td>
<td>8</td>
</tr>
<tr>
<td>Alveolar ridge</td>
<td>11</td>
</tr>
<tr>
<td>Gingiva</td>
<td>11</td>
</tr>
<tr>
<td>Palate</td>
<td>5</td>
</tr>
<tr>
<td>Lip</td>
<td>12</td>
</tr>
<tr>
<td>Vestibule</td>
<td>2</td>
</tr>
</tbody>
</table>
which was moderately dysplastic on biopsy). There were no differences noted regarding visibility or border distinctness between benign and dysplastic lesions. In addition, VELScope examination resulted in the change of the clinical provisional diagnosis of 22 lesions, and changed the biopsy site of 4 lesions.

**DISCUSSION**

The phenomenon of tissue autofluorescence relies on the presence of endogenous fluorophores in cells to produce fluorescent emission in response to exposure to light of a specific wavelength. Tissue autofluorescence reflects the metabolic and biochemical status of cells; therefore, any alterations in these, as occurs during malignant transformation, may result in light scattering and an altered autofluorescence profile. Malignancies are said to demonstrate lower autofluorescence intensities than normal oral mucosa primarily as a result of breakdown of collagen cross-links and blood absorption due to microvascularization and inflammation, and secondarily due to a reduction in flavin adenine dinucleotide, epithelial thickening, and nuclear back-scattering.

Current research on autofluorescence imaging has focused on alterations in autofluorescence within the green light spectrum. Most normal mucosal linings are identical except the vermilion border and dorsal tongue, which are found to be spectrally different. Betz et al examined 36 head and neck tumors using fluorescence imaging and found 34 to exhibit lower autofluorescence intensity when compared to the surrounding normal mucosa. Dysplastic and neoplastic cells displayed loss of tissue autofluorescence and appeared dark green-black in contrast to the pale green autofluorescence emitted by normal tissue. Several studies have assessed the sensitivity and specificity of autofluorescence imaging in differentiating dysplastic and malignant lesions from normal oral mucosa. Betz et al found correct tumor demarcation in 87.8% of cases tested. Gillenwater et al determined 88% sensitivity and 100% specificity in distinguishing abnormal from normal tissue, although the spectroscopic system was different to that of VELScope. Using blue excitation light to excite green fluorescence, and histopathology as the gold standard, Lane et al achieved 98% sensitivity and 100% specificity in discriminating between normal and severely dysplastic or malignant lesions in a pilot study of 44 patients. Despite positive findings regarding the use of tissue autofluorescence in distinguishing normal

<table>
<thead>
<tr>
<th>Category</th>
<th>Diagnosis</th>
<th>COE (correct n, %)</th>
<th>VELScope (correct n, %)</th>
<th>Combined (correct n, %)</th>
<th>Histopathology (correct n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Homogenous leukoplakia/keratosis*</td>
<td>59 (45; 76.3)</td>
<td>38 (30; 78.9)</td>
<td>48 (38; 79.2)</td>
<td>53</td>
</tr>
<tr>
<td>2</td>
<td>Nonhomogenous leukoplakia/suspicious for malignancy†</td>
<td>24 (7; 29.2)</td>
<td>42 (8; 19)</td>
<td>40 (12; 30)</td>
<td>27</td>
</tr>
<tr>
<td>3</td>
<td>Lesions with lichenoid features‡</td>
<td>33 (27; 81.8)</td>
<td>38 (27; 71)</td>
<td>29 (24; 82.8)</td>
<td>32</td>
</tr>
<tr>
<td>4</td>
<td>Others§</td>
<td>2 (2; 100)</td>
<td>0 (0; 100)</td>
<td>1 (1; 100)</td>
<td>6</td>
</tr>
</tbody>
</table>

Abbreviations: COE, clinical oral examination; VELScope, Visually Enhanced Lesion Scope.

Notes: Biopsied lesions diagnosed provisionally with COE alone, VELScope findings alone, and provisional diagnosis after consideration of both the COE and VELScope findings.

Definitive histopathological findings are included as the gold standard for each lesion. Data in parentheses represent the number and corresponding percentage of lesions that were correctly identified based on definitive histopathology (eg, in the case of homogenous leukoplakia, 59 of these were determined by COE alone, 38 by VELScope alone, and 48 with a combination of both examinations. Of the 59 deemed homogenous with COE, only 45 were correctly identified by histopathology. Overall, however, there were 53 lesions that were identified as homogenous leukoplakia/keratosis by definitive histopathology.

Lesions in this category were diagnosed clinically as nonhomogenous leukoplakia or clinically suspicious for malignancy, and histopathologically as either epithelial dysplasia or oral squamous cell carcinoma.

Lesions in this category included chronic hyperplastic candidosis and an eosinophilic granuloma.
Autofluorescence Imaging of Oral Lesions

from abnormal tissue, there have been no studies specifically evaluating the efficacy of VELScope itself on a large cohort of patients referred for investigation of potentially malignant lesions in a prospective fashion. There have been certain case studies that have assessed the usefulness of such a device in patients already known to have a history of dysplasia or carcinoma in situ.19,24 In our current study, we excluded any patient who was known to have oral epithelial dysplasia or squamous cell carcinoma, and only enrolled patients with suspicious oral mucosal lesions in a prospective fashion. Although the use of autofluorescence imaging has been recommended as an adjunct for oral cancer screening, identification of lesions (case finding), selection of biopsy site and tumor margin demarcation, the populations investigated thus far are not representative of the patient mix encountered in general practice, and as such these studies run the risk of test-referral bias.25 Clearly more work needs to be undertaken in a general practice setting, given the results of the current study, to truly test the effectiveness of VELScope in enhancing early detection of potentially malignant oral lesions.

The results of our study show that the use of VELScope enhanced lesion visualization in 34.74% of cases; however, the low positive predictive value (PPV) of 19% indicates that loss of tissue autofluorescence in itself is a poor indicator of the nature of oral mucosal lesions. Loss of autofluorescence was found in dysplastic, malignant, inflammatory, and benign lesions indicating that skill and training is required when interpreting VELScope findings, and that VELScope is unable to accurately differentiate between these in a consistent manner. Blanching of lesions that show loss of autofluorescence is difficult to perform accurately, and in many cases partial blanching is observed which complicates interpretation. Such lesions should be considered as loss of autofluorescence lesions and warrant further investigation (Figure 2). Of the 118 biopsied lesions, 8 lesions were deemed dysplastic even though there was no loss of autofluorescence (8 of 38 lesions in the retained fluorescence group). Similarly there were 10 lesions that were deemed to be dysplastic and 1 OSCC among lesions that lost autofluorescence and blanched completely on application of pressure (11 of 38 lesions in the diascopic fluorescence group).

The information gathered from the VELScope examination changed the provisional diagnosis and some biopsy sites, but the relevance of this to the results cannot be fully articulated and requires further study. Relying on the VELScope to decide on the biopsy site may have impacted the final histopathological diagnosis, and this in itself requires further study. VELScope may be useful at uncovering clinically undetected lesions as it did in our study in 5 such patients (1 of which was moderately dysplastic on biopsy).

On the whole, VELScope tended to overestimate abnormalities as shown by the decreased number of homogenous leukoplakia lesions and the increased number of nonhomogenous leukoplakia/clinically suspicious for malignancy lesions in Table 4. This was mainly a function of loss of autofluorescence coupled with either partial or lack of blanching, and is

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**TABLE 5.** Correlation of conventional oral examination, VELScope examination, and provisional diagnosis after consideration of both the conventional oral examination and VELScope together, compared with histopathological findings (n = 118 lesions).

<table>
<thead>
<tr>
<th></th>
<th>TP, n</th>
<th>TN, n</th>
<th>FP, n</th>
<th>FN, n</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>COE</td>
<td>7</td>
<td>74</td>
<td>16</td>
<td>21</td>
<td>25%</td>
<td>82%</td>
<td>30%</td>
<td>78%</td>
<td>69%</td>
</tr>
<tr>
<td>VELScope</td>
<td>8</td>
<td>57</td>
<td>34</td>
<td>19</td>
<td>30%</td>
<td>63%</td>
<td>19%</td>
<td>75%</td>
<td>55%</td>
</tr>
<tr>
<td>COMB</td>
<td>11</td>
<td>16</td>
<td>29</td>
<td>15</td>
<td>46%</td>
<td>68%</td>
<td>29%</td>
<td>82%</td>
<td>63%</td>
</tr>
</tbody>
</table>

Abbreviations: TP, true positive; TN, true negative; FP, false positive; FN, false negative; PPV, positive predictive value; NPV, negative predictive value; COE, conventional oral examination alone; VELScope, Visually Enhanced Lesion Scope examination alone; COMB, conventional oral examination and VELScope examination.

Notes: Sensitivity (the proportion of positives which were correctly identified) = TP/TP+FN; Specificity (the proportion of negatives which were correctly identified) = TN/TN+FP; PPV; the proportion with positive test results correctly diagnosed) = TP/(TP+FP); NPV (negative predictive value; the proportion with negative test results correctly diagnosed) = TN/(TN+FN); Accuracy (how well a test correctly identifies or excludes a condition = (TP+TN)/(P+N).

Note: A histopathological diagnosis of dysplasia was considered positive (ie, TP) for the presence of disease in all examinations and comparisons.
particularly problematic in cases of oral lichenoid lesions or dysplastic lesions with an inflammatory component.

According to accuracy calculations (Table 5) the operator relying on conventional oral examination alone is more accurate at correctly identifying and excluding dysplasia, compared to VELScope examination alone. Conventional oral examination as undertaken by oral medicine specialists is reasonable at being able to assure patients that they do not have dysplasia (negative predictive value [NPV] = 78%).

VELScope was found to have a low PPV (19%) indicating that further investigations were required and that VELScope alone could not diagnose dysplastic lesions, and the test in itself only identifies up to a third of all dysplastic lesions (sensitivity 30%). However, this was slightly better than conventional oral examination alone. As a detection/visualization tool alone, it was the least able to assure patients that they did not have dysplasia (NPV = 75%) with a specificity of 63%.

Analyses of data utilizing clinical information gained by the operator using conventional oral examination combined with the loss of autofluorescence and blanching findings of the VELScope showed a PPV (29%) very similar to conventional oral examination alone. Moreover, the combined examinations (conventional oral examination and VELScope) resulted in more dysplastic lesions being noted, with the sensitivity rising to 46%. As a detection/visualization tool, it is more likely that together (VELScope findings and operator interpretation) can assure patients that they do not have dysplasia (NPV 82%). ROC space analysis clearly shows that the combination of both device findings and operator interpretation is more beneficial at identifying dysplasia compared to conventional oral examination or VELScope alone, and that VELScope findings (particularly diascopic fluorescence) are not useful in their own right without clinical interpretation. Clinical interpretation is the Achilles heel of this device, and may prove to be its limitation in a general practice setting without further training and limited expertise in dealing with mucosal lesions.

Figure 2 clearly highlights that dysplastic changes can be found even in lesions that do not lose autofluorescence, and equally importantly, in lesions that lose autofluorescence and then blanch on application of pressure (diascopic fluorescence). Clearly more work is required to determine the factors that may explain a false-positive result in the case of homogenous leukoplakia, and a false-negative result in the case of nonhomogenous leukoplakia/cclinical suspicious lesions. These explanations are more important in a general practice setting compared to a specialist setting.

Despite the high false-positive rate, VELScope demonstrates potential for use as a noninvasive direct visualization aid. It has demonstrated usefulness in assessing margins of known dysplastic and OSCC lesions; however, at the moment it can only be considered a clinical adjunct, requiring significant training in its use, and is potentially more suited in a specialist oral cancer clinic instead of a general dental or medical practice, although further studies are indicated in this context. A thorough and systematic visual examination with white light, careful digital palpation, and scalpel biopsy remain the gold standard for patient care.14,33

CONCLUSIONS

In order to improve patient prognosis, early detection and treatment of malignant and potentially malignant oral lesions is essential. VELScope seems to be of use at aiding the visualization of potentially malignant, malignant, and inflammatory conditions; however, it cannot accurately or consistently differentiate between them, even in the hands of experienced oral medicine specialists. VELScope is a useful clinical tool for visualizing abnormalities of the oral mucosa, but cannot provide a definitive diagnosis as to the presence or otherwise of dysplastic tissue change. Its use requires a significant understanding of mucosal pathology, and interpretation of results requires skill and training. Although clinical inspection and histopathology have certain limitations, they remain the gold standard for determining a definitive diagnosis.

REFERENCES

Effectiveness of autofluorescence to identify suspicious oral lesions—a prospective, blinded clinical trial

Felix Peter Koch · Peer W. Kaemmerer · Stefan Biesterfeld · Martin Kunkel · Wilfried Wagner

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Abstract Regular screening through white light inspection of the entire oral mucosa is the most important examination method to identify precancerous lesions and early oral carcinoma. Additionally, the physiologic autofluorescence of the oral mucosa has been described as a novel screening method for the detection of mucosal lesions that are not visible by white light. This study aimed to evaluate the sensitivity and specificity of the autofluorescence examination. Seventy-eight patients were examined in this study. All of them suffered from suspicious oral mucosal lesions. Two different investigation methods were applied: the standard examination by white light and an examination by a novel light source of 400 nm that evoked a green light emission (>500 nm) in normal mucosa. It was proposed that malignant oral mucosal lesions show different autofluorescence characteristics than the green autofluorescence of healthy mucosa. Red autofluorescence indicated SCC with a sensitivity of 20% and a specificity of 98%. The results showed that dysplasia and carcinoma could be identified with a sensitivity of 96% and a specificity of 18% by using the autofluorescence method. The sensitivity decreased according to the grade of mucosal keratosis and was influenced by the localisation of the lesion. In conclusion, benign as well as malignant oral lesions could not be distinguished by a diminished autofluorescence signal. A red autofluorescence signal, however, could indicate cancerous processes of the oral mucosa.

Keywords Autofluorescence · Prevention · Minimal invasive · Oral cancer · Diagnostic · Clinical trial

Introduction

Cancer located in the mouth or oropharynx concerns 300,000 patients worldwide [1]. The prognosis decreases with advanced cancer stage [2–4], and the therapy of advanced cancer often leads to social stigmatization, speech handicap, and nutrition problems [5–8]. Therefore, early diagnosis of oral carcinoma is crucial for the patient’s benefit. In the past, several minimally invasive diagnostic methods for early diagnosis of oral precancerous or malignant lesions have been published [9–17]. These techniques are based on visual as well as cytological principles. Examples include fluorescence or toluidine blue staining and methods for differential diagnosis such as the brush biopsy and consecutive image cytometry, immune cytology, or gene expression analysis [12, 14, 15, 18–20].

The autofluorescence technique used in this clinical trial is a new, commercially available screening instrument to detect suspicious oral lesions.
Physiologically, the oral mucosa shows a characteristic autofluorescence signal of >500 nm if excited by light of 400 nm [21]. Treatment by florescent chemicals is not necessary. Squamous cell carcinomas (SCCs), however, are supposed to be characterized by a different autofluorescence signal [22, 23]. These observations have been obtained by several studies and different wavelengths [21, 22, 24]. Svistun et al. achieved the best sensitivity and specificity for distinguishing cancer or dysplasia from normal mucosa at an excitation wavelength of 400 or 440 nm and a fluorescence observation at 530 nm, as done in the presented study [25]. They analyzed several regions of three resected carcinoma and one dysplasia using white light, autofluorescence, and incision biopsy, followed by a subsequent histopathologic analysis. They found a sensitivity of 100% and a specificity of 83% for the detection of cancer. Lane et al. examined 50 oral lesions to evaluate the accuracy of the autofluorescence in distinguishing SCC and carcinoma in situ from normal mucosa. They reported a significant correlation of malignant lesions with a lower intensity autofluorescence signal [26].

The differential diagnosis of inflammatory diseases such as lichen planus, severe periodontitis, or posttraumatic inflammation was not addressed in these studies.

Since the autofluorescence extinction of the oral mucosa served as a screening instrument to detect invisible lesions, there currently just exist data on the sensitivity. The potency to differentiate benign and malignant lesions has not been evaluated. The clinician, however, needs an examination instrument that supports the clinical diagnostics and the decision on how to treat a detected lesion. Therefore, data on autofluorescence specificity are urgently needed, but not available. This study aims to evaluate the effectiveness of the autofluorescence investigation and the capability to differentiate between suspicious and benign oral lesions, dysplasia, and SCC.

Material and method

Material

For standard screening of the oral mucosa using white light, the dental chair examination light was used (15V/150W, 64634 HLX OSRAM, Munich, Germany). The light source for autofluorescence excitation (Velscope™, Rocker&Narjes GmbH, Köln) emitted blue light at a wavelength of 400 nm. A dichroic mirror provided coaxial excitation and emission pathway. The autofluorescence was detected at >500 nm by the emission filter, which allowed the green–red fluorescent light to pass and rejected the blue excitation light. Another notch filter divided the fluorescent light spectrum into red and green components. For documentation and blinded evaluation, the oral lesions were photographed with a digital reflex camera by different light sources (Canon EOS 100 clinical white light documentation, Nikon 50 and ISO 1400 for autofluorescence documentation). To record the intensity of autofluorescence, the camera was directly connected with the fluorescence light source so that the perspective, including refraction and wavelength, matched the examiner’s view.

Method

To be included in the study, a mucosal lesion of the oral cavity was required that had been clinically diagnosed as SCC or suspicious epithelial lesions requiring histological evaluation for definitive diagnosis. Patients with clinically healthy mucosa were excluded. The 78 patients participating in the study attended the outpatient clinic of the Oral and Maxillofacial Surgery clinic of the Mainz University Medical Centre and suffered from suspicious oral mucosal lesions. Two different investigation methods were applied: the standard examination by white light and the examination by a 400-nm wavelength light source that is supposed to trigger a green light emission (>500 nm) in normal mucosa. After documentation by digital reflex photography, the suspicious lesion was anesthetized (UDS 1:200.000, Aventis Pharma, Bad Soden, Germany), and a biopsy by incision was performed. Then, the biopsies were fixed with formaldehyde 4.5% (Roti-Histofix, Carl Roth GmbH+CKG, Karlsruhe, Germany) and processed for light microscopy via paraffin-embedded, haematoxylin–eosin-stained slices. All of these investigations were performed by the same investigator.

The photographs of the standard and autofluorescence examinations were evaluated independently and blindly by two different examiners who categorized the white and the autofluorescence aspect of the lesions. Using white light, the visual aspects of a plain leukoplakia, a verrucous leukoplakia, an erythroplakia, an erythroleukoplakia, an ulcer, a completely fibrin-covered lesion, a partially fibrin-covered ulcer, a partially fibrin-covered erythroleukoplakia, as well as a verrucous, erythematous partially fibrin-covered lesion were distinguished.

The clinical white light examination was conducted by one clinician who specialized in oral oncology. These findings were classified as (1) “abnormal but innocuous” (clinically explainable conditions like inflammation, scar, cheek bite, prosthesis incongruence, etc.), and (2) “suspicous for premalignant or malignant lesions”.

The autofluorescence photographs were categorized according to black, dark green, bright green, red, speckled red/black, as well as a speckled green/black aspects (Figs. 1 and 2).
These visual aspects were matched afterwards with the histopathological diagnoses of the scalpel biopsies. The diagnoses of mucosal hyperkeratosis, dysplasia, lichen planus, inflammation, healthy mucosa, dysplasia, and SCC were distinguished.

The sensitivity, specificity, positive and negative predictive values to diagnose SCC, and dysplasia were calculated depending on two different autofluorescence features:

(1) A low or absent autofluorescence signal (black or dark green aspect), as well as red autofluorescence signal, was evaluated as an indicator for dysplasia or SCC (positive). Also, a speckled, heterotopic aspect of both green and autofluorescence negative or reddish regions indicated a positive finding.

(2) The presence of red mucosal autofluorescence was evaluated as a separate indicator for dysplasia or SCC (positive).

Furthermore, the clinical diagnoses were evaluated by cross table analysis and the sensitivity, specificity, positive, and negative predictive values were calculated as well.

Using a blinded study design, the influence of the different examiners was minimized. The effect of the clinical aspects, as hyperkeratosis or hyperemia, and the localization of the lesion on the autofluorescence characteristics have been demonstrated by cross tables. The sensitivity and specificity were evaluated.

The statistical evaluation was performed using the SPSS software (SPSS 15.0 for Windows, SPSS Inc., Chicago, USA).

Fig. 1 Examples of fluorescence classification: a, b autofluorescence extinction (white light aspect normal mucosa, histology healthy mucosa); c, d low autofluorescence signal (white light aspect leukoplakia, histology oral lichen planus); e, f physiological autofluorescence signal (white light aspect leukoplakia, histology oral lichen planus)
Results

The 78 patients in this study had an average age of 61.7 years and 59% of them were males.

Forty-one percent of the oral lesions showed red features like the erythroplakia (17%) or the erythroleukoplakia (24%). A white, hyperkeratotic feature like the leukoplakia was found in 21% of the cases. An ulcerous aspect was described in 21% of the cases, and in 17%, a speckled aspect was found, including fibrin-covered lesions.

The histology results identified 14% of the lesions as mucosal hyperkeratosis, 33% as oral lichen planus, 9% as inflammation, 4% as dysplasia, and 39% of the oral lesions as a SCC. In 1% of the cases, normal mucosa was histologically found, although an erythematous aspect has been presented clinically (Table 1).

The accuracy of the clinical diagnosis to identify SCC was evaluated by high sensitivity (97%) and specificity (95.8%) values due to the experience of a specialized examiner (Table 2).

Table 1 Histopathological diagnosis of lesions included

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>Percent</th>
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<tbody>
<tr>
<td>Mucosal hyperkeratosis</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>Lichen planus</td>
<td>26</td>
<td>33</td>
</tr>
<tr>
<td>SCC</td>
<td>30</td>
<td>39</td>
</tr>
<tr>
<td>Inflammation</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Dysplasia</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Healthy mucosa</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Σ</strong></td>
<td><strong>78</strong></td>
<td><strong>97</strong></td>
</tr>
</tbody>
</table>

Fig. 2 Examples of fluorescence classification: a, b speckled green autofluorescence and low autofluorescence signal (white light aspect erythroleukoplakia, histology dysplasia); c, d speckled red autofluorescence and low autofluorescence signal (white light aspect verru- cous) erythroleukoplakia, histology SCC); e, f red autofluorescence (white light aspect ulcer and fibrin, histology SCC)
The blinded autofluorescence analysis revealed complete autofluorescence extinction in 49% (38) cases. In 13% (10) of the lesions, a physiological green autofluorescence was found. Thirty-eight percent (30) of the lesions were characterized by low autofluorescence, red autofluorescence, or a speckled, heterotopic aspect of both green and autofluorescence negative, as well as reddish regions at the same time.

The findings were reproducible by two different investigators in a blinded study design. Following the definition (1) of positive findings, cross table calculations showed a sensitivity of 93% and a specificity of 13–17% in identifying SCC. The positive predictive value (PPV) was calculated at 41%, the negative predictive value (NPV) at 75–80% (Table 3).

Pooling the histopathological findings of dysplasia and SCC, a high sensitivity and a low specificity were also found (sensitivity, 94%; specificity, 13–18%; PPV, 44–46%; NPV, 75–80%; Table 3).

If only the red autofluorescence findings were used to diagnose SCCs, according to definition (2), the sensitivity was 18–21%, the specificity 98%, the PPV 86–88%, and the NPV 62–63% (Table 3). If the histopathological diagnoses of SCC and dysplasia were pooled, they were identified with a low sensitivity and high specificity using red autofluorescence (Table 3).

Taking the results of the clinical and the autofluorescence examinations together, the sensitivity to identify SCCs could not be improved because the hyperkeratotic SCC that was not diagnosed clinically did not show any autofluorescence abnormalities either.

Looking at white light aspects and their autofluorescence signals, 77% of the oral lesions that showed a physiological autofluorescence of green light had a leukoplakia-like aspect. The sensitivity of diagnosing hyperkeratotic SCC correctly was 50% (Table 4).

Table 5 presents the autofluorescence characteristics and their anatomical localization. Particularly, the dorsum of the tongue did not show autofluorescence extinctions, although two SCCs had been diagnosed in this area by means of histology. Another cancerous lesion of this region could be identified by a red autofluorescence pattern.

### Discussion

This study evaluated the intensity and quality of the emitted autofluorescence signal of >500 nm after excitation by 400 nm, and included 78 suspicious inflammation lesions, mucosal hyperkeratosis, lichen planus, dysplasia, and SCC. Taking all lesions of a deviated autofluorescence signal as positive for SCC, a sensitivity of 93% and a specificity of 13–17% were found (definition (1)). Evaluating only clinically erythematous features, such as dysplasia, lichenoid lesions, or inflammation, the autofluorescence diagnosis led to a false positive result in 59% of these cases (PPV, 41%; Table 3). Erythematous, benign lesions could, therefore, not be distinguished from SCC by autofluorescence.

For red autofluorescence, the PPV was 84–88%; the sensitivity to distinguish SCC from all other lesions, however, was only 18–21%, the specificity, 98%, and the NPV, 42–43% (definition (2)). Therefore, lesions showing a red autofluorescence signal should need further clarification via histology, indicated by a high PPV and a high specificity value.

These results suggest that autofluorescence could help to identify any type of pathological oral lesions using lower fluorescence signal, but could not reliably distinguish benign oral lesions from dysplasia or SCC.

The property of the autofluorescence technique to detect oral lesions that are difficult to identify by white light has already been demonstrated by Huff et al. and is accepted [27]. Several other studies, however, have claimed that fluorescence analysis is highly sensitive for identifying malignant mucosal lesions in the oral cavity [26, 28]. These excellent test results could be caused by a study population

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCC</td>
<td>96.6</td>
<td>95.8</td>
<td>93.5</td>
<td>97.9</td>
</tr>
<tr>
<td>SCC/dysplasia</td>
<td>93.8</td>
<td>97.8</td>
<td>96.8</td>
<td>95.7</td>
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</table>

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>b</td>
<td>a</td>
<td>b</td>
<td>a</td>
</tr>
<tr>
<td>SCC</td>
<td>93</td>
<td>20 (18–21)</td>
<td>15 (13–17)</td>
<td>98</td>
</tr>
<tr>
<td>SCC/dysplasia</td>
<td>94</td>
<td>22 (20–23)</td>
<td>16 (13–18)</td>
<td>98</td>
</tr>
</tbody>
</table>
of completely obvious malignant or benign findings of SCCs and healthy mucosa. Suspicious inflammation of the oral mucosa or oral lichen planus has not been included. However, to evaluate the clinical relevance of fluorescence analysis, these differential diagnoses have to be investigated.

As done by the presented study, also Jayaprakash et al. investigated the autofluorescence characteristics of oral lesions, identified by white light examination. They reported a sensitivity of 80% to identify cancer by white light examination, which is comparable to the results of our study, showing 96.6%. They conducted a loss of autofluorescence to identify suspicious oral lesions. By this autofluorescence algorithm, a test sensitivity of 93.3% to identify cancer and 96% to identify cancer, as well as high-risk-lesions, were described. If white light examination and autofluorescence examination were taken together, all cancer and high-risk lesions had been identified correctly [29].

Our results, however, could not support the additional diagnostic help of autofluorescence application. No cancerous lesion that was clinically not identified was found by the aid of the autofluorescence technique. The influence of lesion characteristics and lesion localization on autofluorescence characteristics, as well as the red autofluorescence, has not been concerned by Jayaprakash et al. [29].

The strong concordance of physiological green fluorescence and the hyperkeratosis of the lesion support the assumption that hyperkeratotic lesions could elude autofluorescence detection. Concordantly, Betz et al. found lesions easier to detect if they were not verrucous or exophytic [30, 31]. Also, concordantly, these authors found a limited assessment of the dorsum of the tongue [31]. No lesion localized at the dorsum of the tongue showed autofluorescence extinction, although two of these lesions of green autofluorescence turned out to be invasive carcinoma after histological diagnosis (Table 5). Concerning hyperkeratotic oral lesions or lesions localized at the dorsum of the tongue, these results suggest a limited benefit for cancer screening by means of loss of autofluorescence.

The exact mechanisms underlying alteration in epithelial autofluorescence remain unclear. Several fluorophores and chromophores which could absorb the autofluorescence signal, as well as an altered tissue structure, could influence the overall optical signals. Fluorophores that emit light at >500 nm are ceroid and eosinophile Granula, amino acids such as tryptophan, and also NADH and oxidized FAD. These coenzymes of the oxidative phosphorylation and glycolysis are altered in the case of malignant mutation as well as inflammation. An influence of inflammation on autofluorescence signal therefore seems feasible, as shown by this study, although Svistun proposed that inflammation did not influence the autofluorescence characteristics [25]. The source of red autofluorescence could be caused by porphyrin that is a typical product of bacterial metabolism. If this were the case, red autofluorescence would not be an appropriate indicator for early diagnosis of SCC or dysplasia [30]. Other fluorophores such as ceroid, however, could also show red autofluorescence and are also being considered.

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Diagnostic effectiveness to identify SCC of hyperkeratinized and reddish aspect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity (%)</td>
</tr>
<tr>
<td>Hyperkeratosis</td>
<td>50</td>
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<tr>
<td>Erythema</td>
<td>92</td>
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<table>
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<tr>
<th>Table 5</th>
<th>Cross table of anatomical region and autofluorescence signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autofluorescence</td>
<td>Total</td>
</tr>
<tr>
<td>No signal</td>
<td>Low signal</td>
</tr>
<tr>
<td>Region</td>
<td></td>
</tr>
<tr>
<td>Cheek</td>
<td>11</td>
</tr>
<tr>
<td>Gingival</td>
<td>15</td>
</tr>
<tr>
<td>Floor of the mouth</td>
<td>3</td>
</tr>
<tr>
<td>Sulcus glossoalv.</td>
<td>2</td>
</tr>
<tr>
<td>Tongue lower side</td>
<td>0</td>
</tr>
<tr>
<td>Tongue dorsum</td>
<td>0</td>
</tr>
<tr>
<td>Palate</td>
<td>3</td>
</tr>
<tr>
<td>Arcus palatogloss.</td>
<td>3</td>
</tr>
<tr>
<td>Inner lips</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
</tr>
</tbody>
</table>
The proposed benefit to detect many invisible, possibly malignant lesions is challenged by the necessity to find a definitive diagnosis of these mucosal lesions. Considering our study results, the autofluorescence does not support the examiner in terms of further therapy decisions because the autofluorescence is not capable to distinguish benign and malignant mucosal lesions. The low test specificity of the autofluorescence screening does not justify an invasive diagnostic effort. In case of clinically suspicious oral lesions, minimal invasive methods should be applied then.

**Conclusion**

With a high sensitivity and NPV, but a low specificity and PPV, oral mucosal lesions could be detected by autofluorescence. The autofluorescence examination, however, is not able to differentiate between benign and malignant oral lesions. Red autofluorescence should be an indication for scalpel biopsy due to a high PPV for cancer.

**Acknowledgement** This study was supported by Rocker&Narjes, who supplied the Velscope® fluorescence light source. Prof. Al-Nawas supplied the prototype Velscope® fluorescence light source.

**Conflict of interest** The authors confirm that they have no conflict of interest.

**References**


Clinical evaluation of an autofluorescence diagnostic device for oral cancer detection: a prospective randomized diagnostic study
Majeed Ranaa, Antonia Zapfb, Marco Kuehlea, Nils-Claudius Gellricha and André M. Eckardta

The prognosis for patients with oral squamous cell carcinoma remains poor despite advances in multimodal treatment concepts. Early diagnosis and treatment is the key to improved patient survival. A device (VELscope) that uses autofluorescence technology, allowing direct fluorescence visualization of the oral cavity, might be a useful tool for oral cancer detection or as an adjunct to standard clinical examination. A total of 289 patients with oral premalignant lesions were randomly divided into two groups for clinical examination of precancerous oral lesions. In group 1, 166 patients were examined conventionally with white light, and in group 2, 123 patients were examined with the autofluorescence visualization device (VELscope) in addition to the white light examination. Biopsies were obtained from all suspicious areas identified in both examination groups (n=52). In the first step, baseline characteristics of the two groups (only white light vs. white light and VELscope) were compared to exclude selection bias. In the second step, for the group examined with white light and VELscope (123 patients), the diagnostic strategies were compared with regard to sensitivity and specificity using biopsy as the gold standard. The results showed that using the VELscope leads to higher sensitivity (100% instead of 17%), but to lower specificity (74% instead of 97%). Thus, we can conclude that the VELscope is a useful new diagnostic device for detection of oral cancer diseases. European Journal of Cancer Prevention 00:000–000 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.

European Journal of Cancer Prevention 2011, 00:000–000

Keywords: autofluorescence diagnostic test, biopsy, detection, oral cancer, VELscope

Introduction
Oral and oropharyngeal cancer is a significant health problem throughout the world. It is the eighth most common cancer worldwide with 300,000 new cases reported every year (Parkin et al., 2005). Many countries feature incidence rates of oral cancer that vary in men from one to 10 cases per 100,000 population (Stewart and Kleihues, 2005). Developing countries suffer from higher incidence rates of oral cancer compared with developed countries (Petersen, 2003). It is a cause for worry that the incidence of the disease is reportedly increasing in most countries, such as central and eastern Europe and the USA (Petersen, 2003; Stewart and Kleihues, 2005). The 5-year overall survival rate for patients with oral cancer has been stagnating for the last 20 years (Bray et al., 2002). The survival rate is only 54% in industrial countries, one of the lowest rates of all major cancers. Five-year survival rates in developing countries barely reach 30% (American Cancer Society, 2005). Smoking (Newcomb and Carbone, 1992) and immoderate consumption of alcohol (Merletti et al., 1989), and human papillomavirus are the main risk factors for oral cancer. The association of the two main risk factors has a synergic effect, boosting the risk of developing oral cancer by 30 times (Blot et al., 1988). Most of the oral carcinomas develop from oral premalignant lesions, particularly leukoplakia, erythroplakia, and lichen planus (Scheifele and Reichart, 2003). According to literature data, premalignant lesions might turn into carcinoma in a percentage varying between 5 and 18% of cases; hence early identification of potentially malignant disorders is important to prevent the onset of tumors (Lumerman et al., 1995). Early diagnosis of tumor significantly increases survival rates and reduces impairment of health and quality of life through surgical therapy (Burzynski et al., 1997; Howaldt et al., 1999; Palmer and Grannum, 2011). Nevertheless, most oral carcinomas are currently detected at a late stage. The main reason for this delay is not only the lack of awareness of the symptoms and risk factors among the public (Mashberg, 2000) but also the lack of prevention and early detection by healthcare providers (Mignogna et al., 2001). Early detection is also impeded by the lack of typical clinical characteristics, such as ulceration, induration, or pain at early carcinoma stages (Mashberg and Samit, 1995). Early malignant lesions are often indistinguishable from normal-looking mucosa, making them harder to detect even for experienced examiners (Shugars and Patton, 1997). Currently,
biopsy is considered as the gold standard for the diagnosis of oral carcinoma, because the grade of epithelial dysplasia can only be diagnosed in a histopathological specimen (Natarajan and Eisenberg, 2011). The standard method for oral cancer screening is a conventional oral examination (COE) using normal (incandescent) light (Patton et al., 2008). Numerous publications indicate that COE may have limited value as a method for detecting precancerous lesions (Silverman, 1988; Downer et al., 2004). Additional screening aids for improving the detection rates of oral cancer are needed and are being marketed by the industry. There are several studies assessing the diagnostic value of the different new diagnostic methods (Lingen et al., 2008; Patton et al., 2008; Fedele, 2009; Trullenque-Eriksson et al., 2009; Seoane Leston and Diz Dios, 2010). Toluidine blue is a vital dye to improve the visibility of lesions during visual examination, but it has a relatively low specificity. The technique was reviewed in the literature with data for sensitivity ranging from 38 to 98% and specificity ranging from only 9 to 93% (Patton et al., 2008; Epstein and Guneri, 2009). Another visual adjunct for oral examination is the ViziLite from Zila Pharmaceuticals Inc. (Phoenix, Arizona, USA). Blue light emitted by a disposable chemiluminescent light source illuminates the oral tissue, apparently improving the brightness and sharpness of oral premalignant lesions (Epstein et al., 2006). However, some studies concluded that examination with the ViziLite did not change the diagnosis (Ram and Sier, 2005; Farah and McCullough, 2007). The use of autofluorescence imaging is a similar noninvasive approach for improving the detection of potentially malignant oral cavity lesions (Lane et al., 2006). As these systems do not represent a complete diagnostic device, they have to be supplemented by additional hardware devices. In consequence, the handling of these systems is of an experimental nature, and the detection of oral malignant lesions is not feasible in daily routine. The VELscope System by LED Medical Diagnostics (White Rock, British Columbia, Canada; VELscope: the Oral Cancer Screening System, LED Dental Inc., Burnaby, British Columbia, Canada), a novel fluorescence technology allowing direct fluorescence visualization of the oral cavity, might be a useful tool. The purpose of this clinical study was to establish and clinically evaluate a novel, user-friendly diagnostic device for oral cancer prevention as an adjunct to standard clinical examination in a clinical setting with regard to the sensitivity and specificity of the autofluorescence examination in comparison with COE alone.

Materials and methods
The study was approved by the local ethics committee at the Hannover Medical School, Germany (EK 5586/2009). Study participants were enrolled in a clinical protocol reviewed and approved by the institutional cancer board. Before beginning the study, written informed consent was obtained from each patient.

Patients
Patients were enrolled from the Hannover Medical School, Department of Craniomaxillofacial Surgery. A total of 289 patients with oral premalignant lesions were randomly divided into two groups for clinical examination of oral cancer lesions (COE). In group 1, 166 patients were examined with conventional white light, and in group 2, 123 patients were examined with an autofluorescence visualization device, VELscope (autofluorescence visualized examination), in addition to the white light examination. Biopsies were obtained from all suspicious areas identified in both examination groups ($n = 52$).

Study inclusion criteria and protocol
Only patients with an oral premalignant lesion (leukoplakia, erythroplakia, lichen planus, or pemphigus vulgaris) were included in this study. Potential participants were excluded from the study if they had a history of oral cancer or cancer recurrence, possibility of missing follow-up examination, were pregnant, nursing, had undergone recent operations, or had diseases of the heart and circulation, infections, systemic and malignant diseases, or immune system-affecting diseases, or blood coagulation disorders and allergic reactions to pharmaceuticals and antibiotics. The clinical inclusion and exclusion criteria are shown in Table 1.

All patients provided informed consent and completed a detailed questionnaire, which included information on demographics, smoking and alcohol use, current medications, and general health and dental care history. Oral health-related quality of life was evaluated for all patients of completing the abbreviated German version of the Oral Health Impact Profile (OHIP-G-14). A lesion protocol based on the topographical classification system of Roed-Petersen and Renstrup (1969) was applied. All patients were examined using standardized methods and techniques. The visual and VELscope examination was carried out by experienced examiners. To avoid bias, patients

<table>
<thead>
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<th>Table 1 Study inclusion and exclusion criteria</th>
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<tbody>
<tr>
<td><strong>Inclusion criteria</strong></td>
</tr>
<tr>
<td>Oral premalignant lesion: leukoplakia, erythroplakia, lichen planus, or pemphigus vulgaris</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>18–75</td>
</tr>
<tr>
<td>Written informed consent</td>
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were examined in two different examination rooms, and the examiner using the VELscope was unaware of the results of the conventional group.

Conventional method for oral cancer screening
For conventional oral cancer screening of the oral cavity, a dental chair examination light was used (15V/200W, OSRAM, OSRAM AG, Munich, Bavaria, Germany). The standard clinical examination includes visual inspection of the oral mucosa, followed by palpation of suspicious lesions. Initial clinically abnormal lesions were noted and photographed with a digital reflex camera (Pentax *ist DS, Pentax Imaging Systems GmbH, Hamburg, Germany) equipped with a Pentax 100-mm Macro Lens and Macro Ring Lite. After photo documentation the suspicious lesion was biopsied under local anesthesia. All specimens were placed in 4% buffered formalin for fixation and sent for histopathological examination. The presence or absence of dysplasia in the biopsy specimen was recorded by an experienced oral pathologist.

Additional autofluorescence examination using the VELscope
The additional autofluorescence visualised examination was carried out in a dark environment using the VELscope V2 device supported by Mectron Inc. (Cologne, Germany). Patients wore protective glasses during the entire examination. Suspected lesions were photographed using the above-mentioned camera equipment without the ring flash being mounted on the back of the hand piece using an adapter (Photo Med VELscope Photography System, Photomed International, Los Angeles, California, USA). After photo documentation and noting of lesions, biopsy was taken in the above-mentioned manner. To reduce the rate of false-positive results, a follow-up visit 2 weeks after the first examination was implemented if there was any suspicion that the lesion was of acute inflammatory origin. Possible causes of inflammation (sharp teeth, edges of insufficient fillings, poorly fitting set of dentures, etc.) were eliminated by then. Persisting lesions required a biopsy (Thumfart et al., 1978). Following the manufacturer’s advice (LED Dental Inc., 2009), a diascopy (Rudd et al., 2001) was performed on any suspicious lesion to reduce the rate of false-positive results. Applying soft pressure with a clear tongue depressor may restore normal autofluorescence in inflammatory lesions by reducing the pathologically increased blood flow (LED Dental Inc., 2009). Fluorescence loss in malignant or premalignant lesions is not modified by this test.

VELscope device
The VELscope is a device for the direct visualization of changes in tissue fluorescence in the oral cavity. It consists of a bench-top casing containing a 120 W metal-halide arc lamp plus a system of filters and reflectors optimized for producing near-ultraviolet/blue light between 400 and 460 nm and a coupled handheld unit for direct observation (Lane et al., 2006). If needed, a camera can be connected to the hand piece for the purpose of documentation. Digital image processing of wide-field autofluorescence images can be used to outline suspicious regions in real time. The autofluorescence observed in wide-field images of the normal oral mucosa originates primarily from stromal collagen. Oral neoplasia is associated with a loss of stromal autofluorescence. Benign lesions, such as inflammation, are also associated with loss of stromal autofluorescence, which may limit diagnostic specificity, especially in low-risk populations.

Technique of autofluorescence visualization
The autofluorescence of tissue and its potential use in cancer detection were described first in 1924 (Policard, 1924). Naturally occurring fluorochromes (e.g. collagen, elastin, keratin, FAD, NADH) (Richards-Kortum and Sevick-Muraca, 1996) that are located in the epithelial cell lining and submucosa of the oral cavity show fluorescence in the green spectral range when excited with light between 375 and 440 nm (Betz et al., 1999). Malignant or dysplastic alteration causes complete loss of the normal tissue fluorescence (fluorescence visualization loss) because of the disturbance in the distribution of these fluorochromes (Svistun et al., 2004; Lane et al., 2006). According to the literature, autofluorescence spectroscopy has a sensitivity and specificity higher than 95% for differentiating malignant tumors from healthy oral tissue. Adding autofluorescence imaging to conventional clinical examination could possibly improve sensitivity and specificity (Kulapaditharom and Boonkitticharoen, 2001; Betz et al., 2002). Recent studies have criticized the failure of the VELscope to discriminate high-risk lesions from low-risk lesions (Awan et al., 2011) and its high rate of false-positive results (Balevi, 2007).

Statistical analysis
The sample size for the study was planned using the data of a pilot study (n = 30). In this pilot study, the white light examination showed a sensitivity of 50% and a specificity of 100% and for white light plus VELscope the result showed a sensitivity of 100% and a specificity of 96%. The aim of the study was to prove that, with the additional use of the VELscope, the sensitivity is significantly higher and the specificity is not relevantly lower (a loss of specificity of more than 20% is considered relevant). Because both hypotheses had to be rejected for the success of the study, type one error did not have to be adjusted (two-sided 5%); however, the power had to be set to 90% for each hypothesis. With an assumed incidence of 10%, this led to a sample size of 150 patients.

Statistical analyses were carried out using SPSS for Windows version 18.0 (SPSS Inc., Chicago, Illinois, USA). In the descriptive analysis for quantitative variables, boxplots were drawn to decide whether normal distribution could be assumed. If the distribution was symmetric,
mean and SD were calculated, and the two-sample *t*-test was used for comparison between the groups. If the distribution was nonsymmetric, the median (minimum and maximum) was calculated and the Mann–Whitney *U*-test was used for the two-group comparison. For categorical variables, absolute and relative frequencies were calculated and the *χ²*-test and Fisher’s exact test were used for comparison, respectively.

In the first step, the two groups (with or without the additional use of the VELscope) were compared with the above-described descriptive analyses. Afterwards, for the group with additional use of the VELscope, the baseline characteristics and the OHIP score were analyzed descriptively for the patients with and without cancer lesions.

For the primary analysis, the differences (white light plus VELscope vs. white light only) in the sensitivities and specificities of the two diagnostic approaches, with the corresponding two-sided 95% Agresti confidence intervals, were calculated. Superiority with regard to sensitivity was concluded if the lower limit of the corresponding confidence interval was above 0, and noninferiority with regard to specificity was concluded if the lower limit of the corresponding confidence interval was above –0.2. As a secondary analysis, the sensitivities and specificities with the corresponding two-sided Agresti confidence intervals were calculated for the two tests separately.

### Results

Because of time restrictions in the daily diagnostic process, only 123 of 269 patients fulfilling the inclusion and exclusion criteria could be examined with the VELscope additionally. The selection of the patients for this group was determined randomly on the basis of the availability of the VELscope, and there were differences between the two groups regarding alcohol intake and frequency of biopsy (Table 2).

In contrast to the assumption for the sample size calculation, the incidence in the population was 5% instead of 10%. This led to six patients with cancer lesions and 117 patients without cancer lesions. The two groups (with vs. without cancer lesion) were compared descriptively. The alcohol intake of patients with and without cancer lesions was different; however, the other parameters were distributed similarly in the two subgroups (Table 3).

The results of the evaluation of the diagnostic accuracy are shown in Table 4. As expected, the additional use of the VELscope led to a higher sensitivity (100% instead of 74%), but to lower specificity (74% instead of 97%) (Figs 1–3).

The loss of fluorescence in all examined lesions is shown in Table 5.

### Discussion

Early detection of oral cancer is one of the most efficient ways to reduce the high mortality due to this disease. It can minimize the morbidity of the disease and its treatment, which is associated with a severe loss of function, disfigurement, depression, and poor quality of life. There is increasing demand for additional useful tools for cancer detection to supplement conventional white light oral examination (Balevi, 2007). Our study evaluated the diagnostic accuracy of the VELscope device. In conclusion, the additional use of the VELscope

---

**Table 3 Baseline characteristics of the two subgroups (with VELscope, with or without cancer lesion)**

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<th>With cancer lesion</th>
<th>Without cancer lesion</th>
<th><em>P</em>-value</th>
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<tr>
<td><strong>Age, mean ± SD</strong></td>
<td>58 ± 9</td>
<td>63 ± 11</td>
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<td><strong>OHIP score, median (min–max)</strong></td>
<td>3 (0–15)</td>
<td>4 (0–32)</td>
<td>0.511</td>
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<td><strong>Sex, n (%)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Male</td>
<td>4 (67)</td>
<td>42 (36)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>2 (33)</td>
<td>75 (64)</td>
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<tr>
<td><strong>Smoking, n (%)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>1 (17)</td>
<td>21 (19)</td>
<td>1.000</td>
</tr>
<tr>
<td>Previous</td>
<td>2 (33)</td>
<td>31 (27)</td>
<td></td>
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<tr>
<td>Actual</td>
<td>3 (50)</td>
<td>61 (54)</td>
<td></td>
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<tr>
<td><strong>Alcohol, n (%)</strong></td>
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<tr>
<td>Never</td>
<td>3 (50)</td>
<td>43 (37)</td>
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<td>≤ 20 g/day</td>
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<td>40 (34)</td>
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<tr>
<td>21–40 g/day</td>
<td>1 (17)</td>
<td>24 (21)</td>
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<td>41–60 g/day</td>
<td>1 (17)</td>
<td>9 (8)</td>
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<tr>
<td>61–80 g/day</td>
<td>1 (17)</td>
<td>–</td>
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<tr>
<td>Unkown</td>
<td>1 (17)</td>
<td>1 (0.9)</td>
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<td><strong>Frequency of examination, n (%)</strong></td>
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<td>Twice a year</td>
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<td>Once a year</td>
<td>5 (83)</td>
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increased sensitivity from 17 to 100% compared with COE alone in detecting malignant lesions of the oral mucosa but reduced specificity significantly from 97 to 74%. A loss of fluorescence was detected in 100% of all dysplastic lesions, which shows the ability to detect high-risk lesions (Table 5). However, 37.84% of all cases of leukoplakia/erythroplakia and the majority (81.08%) of all clinically diagnosed cases of lichen planus showed loss of tissue fluorescence (Table 5). A recent study by Awan et al. (2011) showed similar results and criticized the lack of specificity of the technique. Our results indicate that autofluorescence examination can help to identify dysplastic lesions but cannot differentiate benign oral lesions such as inflammation or oral lichen from malignant lesions reliably. It is disappointing that 64.23% of all examined lesions showed a loss of fluorescence, whereas only 4.88% of the lesions could be identified as dysplasia. This could lead to overdiagnosis if the VELscope is used by a nonspecialist. In our experience, the findings of the VELscope are very subjective, and both clinical experience and training are needed to accomplish good test results. In our study, the relatively low specificity of the device led to a rather large number of false-positive test results (26 patients with 32 biopsies), although a strict examination protocol was applied for the autofluorescence examination (use of diascopy, follow-up visit). This is not acceptable for clinical purposes. False-positive examination results not only frighten patients but also increase morbidity risks because of unnecessary biopsy. A similar conclusion was made in an up-to-date study by Balevi (2011). The high rate of false-positive test
results was also highlighted by Scheer et al. (2011) in a recent study. There are several studies that support the ability of the VELscope to identify areas of dysplasia (Lingen et al., 2008; Parton et al., 2008). Another study resembling our results for the high sensitivity of the device was published in 2006 by Lane et al. (2006). Using histology as the gold standard, the device achieved a sensitivity of 98% in discriminating normal mucosa from severe dysplasia or carcinoma in situ. Therefore, the author recommended this device as a suitable adjunct for oral cancer screening. This study also showed excellent test results of 100% for the specificity of the device. The clear difference from the mere 74% specificity of our study could be because only high-risk patients with a former oral cancer diagnoses were examined in that study, whereas our study population consisted of patients with different histologic diagnoses. Different studies commended the VELscope for biopsy guidance in superficial lesions of the oral mucosa (De Veld et al., 2005; Kois and Tuelove, 2006). Our clinical experience during the examinations was similar. To conclude, VELscope is a simple, noninvasive examination test of the oral mucosa with the ability to help locate malignant oral lesions and find the right location for a biopsy. However, its results have to be interpreted carefully, and a good examination protocol and documentation is very important to decrease false-positive results. It cannot replace histological evaluation of the oral tissue as a gold standard.

Conclusion

Early diagnosis of oral cancer is a major requirement for multidisciplinary oncologic physicians. Detection should lead to less damage from cancer therapy and to better prognosis. The VELscope device, which uses visible light of 430 nm wavelength to cause fluorescent excitation of certain compounds in the tissues, will play a major part in prevention of oral cancer diseases.

Acknowledgements

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Conflicts of interest

There are no conflicts of interest.

References


Fluorescence Visualization Devices in General Dentistry: Seeing the Big Picture

David C. Morgan, PhD, Chief Science Officer, LED Dental Inc.

Dental professionals have been traditionally limited to the use of incandescent light for the visual inspection of the oral cavity. Direct visualization of reflected white light from mucosal surfaces can enable the detection of gross tissue abnormalities, but may fail to identify some early disease processes (such as dysplasia) that have not yet caused changes easily observed using incandescent light.

The limitations of white light have stimulated the search for alternative modalities, and in 2006, after extensive research, the VELscope system was approved in Canada and cleared by the FDA in the United States. Like subsequent entries in its category, such as the Identifi 3000, and the Sapphire Plus Lesion Detection, the VELscope is a non-invasive, handheld device that allows the direct visualization of oral-cavity fluorescence. There are currently two approved indications for the use of oral fluorescence visualization devices: to help clinicians detect cancerous and precancerous lesions and other lesions that might not be apparent to the naked eye, and to help specialists determine appropriate surgical margins.

The VELscope induces natural tissue fluorescence by illuminating the oral cavity with a bright blue light. The resulting tissue fluorescence is significantly dimmer than the blue excitation light reflected from the tissue, but can be directly visualized by looking through the device’s handpiece, which blocks reflected light and optimizes contrast with filters situated along the viewing path.

Mucosal abnormalities often present with abnormal fluorescence patterns that can aid the user in detecting unhealthy tissue. Decreased tissue fluorescence resulting in abnormal fluorescence patterns arises from a variety of causes, including:

- Increases in metabolic activity in the epithelium.
- Breakdown of the fluorescent collagen cross-links in the connective tissue layer beneath the basement membrane.
- Increase in tissue blood content, either from inflammation or angiogenesis (hemoglobin strongly absorbs fluorescence excitation [blue] and emission light [green]).
- Presence of pigments (e.g. melanin or amalgam particles) which absorb light.

Fluorescence visualization devices are particularly sensitive to dysplasia and cancer, disease processes which often involve the first three of the mechanisms bulleted above. Inflammation, on the other hand, is a common occurrence in the oral cavity and also presents as a strong loss of fluorescence, as will certain normal tissues, usually because of their high vascularity or associated blood content. Clinicians utilizing fluorescence devices should familiarize themselves with the normal appearance and patterns of oral cavity fluorescence. This will better equip them to recognize abnormal patterns when they present.
By definition, the use of an adjunctive device is subordinate to a larger diagnostic picture and should not be thought of as a diagnostic test with a definitive “yes/no” or “positive/negative” answer. To properly understand the significance of the fluorescence examination, it must be considered together with the head and neck visual and tactile exam—which itself is embedded within a larger diagnostic process that includes health history, patient interview, and biopsy when required. A particular fluorescence pattern or loss of fluorescence can mean different things in different clinical contexts. Fluorescence visualization never replaces the clinical judgment of the clinician nor overrules areas of concern discovered by means of the traditional examination. The value of fluorescence visualization lies in the fact that it is based on a different type of interaction with tissue than conventional reflectance of white light, and can therefore show the clinician areas of concern that may have been missed during the white light exam. This can lead to the early discovery of lesions, with consequent benefits: enhanced quality of care provision for the clinician; more effective, less invasive therapeutic intervention for the patient; potential improvement of the patient’s quality of life.

Over the past six years, considerable research has attempted to evaluate the use of fluorescence visualization (predominantly focused on the VELscope system) as an aid for the general dentist and specialist.1-18 In addition, some review articles have attempted to evaluate the general benefits of oral cancer screening, and of adjunctive aids such as VELscope.19-21 This work has encompassed a broad spectrum of applications and methodologies; in particular, there has been excellent research devoted to surgical applications. Some of the research directed towards general use by dentists, however, adopts a narrow vision of the utility of the technology, and often fails to evaluate the device according to its stated indications for use. In particular, many authors compare the use of fluorescence visualization to a head and neck exam as a standalone diagnostic procedure for oral cancer, instead of evaluating the added value of using fluorescence visualization in combination with the head and neck exam for the detection of oral disease. This confusion is puzzling, as fluorescence visualization is intended to be, and is approved as, an adjunctive methodology for the detection of all oral mucosal abnormalities.

There have been some notable exceptions; Huff et al9 conducted an interesting retrospective analysis comparing consecutive years in a private dental practice. During the second year a VELscope examination was added to the head and neck exam and ten dysplastic lesions were detected in the patient population as compared to none in the previous year. Most recently, a 620-patient study at the University of Washington18 demonstrated that the addition of VELscope to routine clinical examinations resulted in the detection of a number of mucosal abnormalities not detected by the conventional exam. These abnormalities included a number of dysplasias, as well as lichen planus and other inflammatory lesions. The study highlights an aspect of fluorescence visualization that is often overshadowed by its role in the detection of oral dysplasia and cancer. Devices such as the VELscope provide general practitioners with a powerful tool to aid in the discovery of most types of oral lesions, such as viral, fungal and bacterial infections; inflammation from a variety of causes (including lichen planus and other lichenoid reactions); squamous papillomas, salivary gland tumours, etc.

CLINICAL EXAMPLES

The following clinical examples have been chosen to illustrate the above concepts.

Figure 1 illustrates an important point — automatically associating a loss of fluorescence with pathology is misguided. Note that the left tonsillar pillars, palatine tonsil and oropharynx are predominantly dark (i.e., show a “loss of fluorescence”) because of absorption of light by the associated presence of vascularity and lymphoid tissue. Not
all individuals, however, show this type of lymphoid aggregate proliferation. With a little experience, one becomes familiar with the spectrum of normal variation present in a wide cross-section of individuals seen in a typical dental practice. Lymphoid aggregates may become uniformly more prominent from inflammatory response; the clinician, however, should pay close attention to unilateral or asymmetrical changes as possibly suggestive of pathological change. The taking of fluorescence and conventional white light photographs, even of normal appearing tissue, facilitates this process by establishing a baseline against which future clinical and fluorescence presentations can be compared. Photographic documentation is an important part of the fluorescence visualization protocol, and is made possible by LED Dental’s newest device, the VELscope Vx, designed to accommodate an optional, custom-built digital camera system.

Inflammatory changes from a wide variety of causes are relatively commonplace. Probably the most common occurrence is trauma-associated inflammation, as seen in this example on the left buccal mucosa (Fig. 2). The subtle visual appearance under white light is transformed, under VELscope, into two dramatic areas of loss of fluorescence that are difficult not to notice. Once seen, the fluorescence response together with the white light presentation paints a consistent picture of the underlying cause. The two dark patches correspond to the two mildly erythematous areas visible under white light. The vessel damage on the upper part of the buccal surface presents predictably as a dark area under fluorescence due to blood absorption, and is consistent with the picture of trauma from the teeth. Rather than being viewed as some sort of “false positive” or distraction, the fluorescence response should help focus the clinician on a legitimate (albeit non-life threatening) possibility of chronic trauma to the buccal mucosa, that may not have otherwise been noticed. This type of trauma can be caused by parafunctional habits, sharp or jagged cusps or malposed teeth, and could be addressed through counseling, oral appliances or smoothing of rough tooth surfaces.

This next case (Fig. 3) illustrates inflammation of a biological, as opposed to traumatic, origin. The patient had a history of asymptomatic red patches on the hard palate for the previous eighteen months. The loss of fluorescence and the erythematous, inflamed appearance under white light led the clinician to suspect candidiasis. Subsequent anti-fun-
gal therapy led to resolution in four weeks as shown below (Fig. 4).

The importance of always considering the results of the physical, visual and tactile examination in the context of the larger clinical picture is highlighted by consideration of the hard palates in these examples (Fig. 5).

Superficially, the cases in Figures 5a & 5b present similarly under both fluorescence and white light illumination, yet when evaluated together with patient history and risk factors, the picture that emerges is significantly different. The first patient complained of a sore mouth and reported sucking on hard candies. The second patient was asymptomatic, but had a number of risk factors (such as tobacco use and age) for the development of oral squamous cell carcinoma. In addition, subsequent follow-up resulted in complete resolution for patient 1, but no change for patient 2, confirming the clinician’s intention to refer the second patient for biopsy, which detected the presence of dysplasia.

The three cases shown in Figures 6, 7 and 8 are all related to lichenoid tissue changes, but each has its own story to tell about the role of fluorescence in the oral mucosal diagnostic process. The first case (Fig. 6) presented with a subtle appearance under white light but demonstrated a striking loss of fluorescence when viewed through the VELscope. In addition to highlighting the presence of the lesion, fluorescence visualization also indicates a much larger area of mucosal involvement than suggested by the white light appearance. The juxtaposition of the lesion to the gold crown suggests a possible allergic lichenoid reaction to the metal, but the final decision regarding causation requires patch testing.

The case in Figure 7 presented clinically as would classic erosive lichen planus; patch testing on the patient failed to reveal any allergic reaction to typical den-
metal restorative materials. Notice how much better visualized the full inflammatory response of the tissue is under fluorescence, as compared to conventional illumination.

The case in Figure 8 initially presented under white light as a classic case of reticular lichen planus; fluorescence highlights the presence of an intense inflammatory response adjacent to the metal restoration on the rear molar, but less so in other regions of the lichenoid reaction. This is clinically significant since the cause of the lichenoid response has a direct bearing on the therapeutic intervention: palliative use of topical steroids to treat the inflammation, as opposed to removing the cause of the allergic lichenoid reaction by replacing the metal restoration.

The distinct and localized loss of fluorescence observed on the hard palate of this patient (Fig. 9) is in striking contrast to the almost complete lack of colour or texture change as observed under white light. Although not evidenced by the white light photograph, there was a palpable bump corresponding to the area of loss of fluorescence. Biopsy confirmed the presence of a salivary gland tumour (low-grade mucoepidermoid carcinoma). This case demonstrates how loss of fluorescence can indicate serious abnormal pathology in the almost complete absence of other visual changes. (It also demonstrates the importance of palpating all oral structures when conducting the intra-oral soft tissue examination.) Note that fluorescence visualization played two roles: as an aid to discovery and to help confirm that this is a suspicious area warranting follow-up.

Another interesting facet of this case is that the lesion was not an epithelial-based cancer but originated from the salivary gland. It is postulated that the loss of fluorescence was caused by disruption of stromal collagen (breakdown of collagen cross-linking) brought about by tumour growth in the connective tissue layer. One might wonder if “benign” growths such as an

---

**FIGURE 8**—Fluorescence visualization helped answer the question — is this classic reticular lichen planus or a lichenoid reaction? (Images courtesy of the University of Washington Oral Medicine Program.)

**FIGURE 9**—This salivary gland tumour is difficult to visualize under white light but stands out under fluorescence visualization. (Images courtesy of Dr. Samson Ng.)

**FIGURE 10**—Fluorescence visualization helps bring this squamous papilloma to the attention of the clinician. (Images courtesy of Dr. Samson Ng.)

**FIGURE 11**—Classic example of an irregular area of loss of fluorescence associated with dysplasia. (Images courtesy of Dr. Samson Ng.)
adenoma cause a loss of fluorescence. In fact, loss of fluorescence is likely to be a feature of both benign and malignant tumours, since both disrupt stromal collagen. An enlightened approach to the utility of fluorescence as a diagnostic tool would not regard this as a limitation, but as a useful feature; benign and malignant tumours require biopsy for definitive diagnosis and both require therapeutic intervention.

Figure 10 illustrates another example of how an unremarkable appearance under white light can correspond to an obvious abnormality with fluorescence. This particular lesion is a squamous papilloma which must be biopsied for definitive diagnosis and then is typically excised. Note that earlier discovery and diagnosis leads to less invasive intervention for the patient.

This somewhat clinically obvious lesion in the floor of mouth (Fig. 11) is a classic example of a large, irregular area of loss of fluorescence corresponding to precancerous dysplasia. Note the highly asymmetric nature of the lesion when viewed through the VELscope, as well as the irregular, well-defined border — an abnormal fluorescence pattern highly suggestive of precancerous or cancerous changes.

This example of a dysplastic lesion on the lateral border of the tongue (Fig. 12) highlights the added clinical value that the VELscope can bring even when the main part of the lesion is clinically obvious. In this case the area of loss of fluorescence extended at least 10-15mm anterior to the main, clinically apparent part of the lesion, and was also biopsy-confirmed as dysplasia.

This final example (Fig. 13) illustrates how the VELscope can bring the clinician’s attention to an area that might otherwise be overlooked. At first sight, this appeared to be a case of denture trauma with inflammation in the vestibule, as well as hyperkeratotic areas apparent on the edentulous ridge. However, the striking loss of fluorescence corresponding to the hyperkeratosis on the ridge (in the absence of any other clinical signs of inflammation) is highly suspicious, and alerted the clinician to biopsy the area. This resulted in a diagnosis of dysplasia.

When fluorescence visualization devices such as the VELscope are used in their proper clinical context, adjunctively and as part of the complete diagnostic protocol, including patient history and the traditional head and neck exam using white light and palpation, general dentistry practices are provided with a new perspective on the health of oral mucosal tissues. Beyond the dramatic and profound benefits of early dysplasia and cancer detection, examination including fluorescence visualization can assist dentists and hygienists in bringing their patients closer to a state of “total oral health,” with its corollary systemic benefits.

David Morgan is Chief Science Officer at LED Dental Inc. and has 15 years of R&D and product development experience in the use of fluorescence as an aid to disease detection.

The author gratefully acknowledges the help of Jeff Keller in the preparation of this manuscript and would also like to thank Drs. Edmond Truelove, Samson Ng and Scott Benjamin for providing...
clinical images and reviewing the manuscript.

Oral Health welcomes this original article.

REFERENCES

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CFL Legend
Michael “Pinball” Clemons

www.oralhealthgroup.com

December 2011

15
Appendix T
**Efficacy of Optically-guided Surgery in the Management of Early-staged Oral Cancer**

*This study is currently recruiting participants.*

Verified on February 2011 by University of British Columbia

First Received on December 22, 2009. Last Updated on February 23, 2011

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**Purpose**

Oral squamous cell carcinoma (SCC) is a global disease responsible for ~300,000 new cancer cases each year. Local recurrence (~30% of cases) and formation of second primary malignancy are common. Cosmetic and/or functional compromise associated with treatment of disease stage is often significant. These statistics underscore the urgent need to develop a better approach in order to control this deadly disease.

It is becoming increasingly apparent that oral cancers develop within wide fields of diseased tissue characterized by genetically altered cells that are widespread across the oral cavity and present in clinically and histologically normal oral mucosa. Complete removal of these lesions is difficult because high-risk changes frequently go beyond clinically-visible tumor. In recognition of this, current best practice is to remove SCC with a significant width (usually 10 mm) of surrounding normal-looking oral mucosa. However, since occult disease varies in size such approach often results in over-cutting (causing severe cosmetic and functional morbidity) or under removal of disease tissue, as evidenced by frequent positive surgical margins and high local and regional recurrence - a failure of the best practice.

There is a wealth of literature that supports the use of tissue autofluorescence in the screening and diagnosis of precancers in the lung, uterine cervix, skin and oral cavity. This approach is already in clinical use in the lung and the mechanism of action of tissue autofluorescence has been well described in the cervix. Changes in fluorescence reflect a complex interplay of alterations to fluorophores in the tissue and structural changes in tissue morphology, each associated with progression of the disease.

As one of the internationally leading teams in applying tissue fluorescence technology, we have shown that direct fluorescence visualization (FV) tools can identify clinically-visible or occult premalignant and malignant lesions that are associated with lesions at risk, with high-grade histology and high-risk molecular change. In a recently completed, prospective study, we have shown that FV helped surgeons in the operating room to determine the extent of the high-risk FV field surrounding the cancer and resulted in remarkably lower 2-year recurrence rates (0% for FV-guided vs. 26% for those without FV-guided approach). There is need to design a larger scale prospective, randomized controlled (Phase III) trial to gather strong evidence in proving the efficacy of the surgery approach using this adjunct tool.

To establish the evidence supporting the change in clinical practice using FV-guided surgery. There are 3 objectives.

1. **Objective 1 (Clinical evidence):** To assess the effect of FV-guided surgery on the recurrence-free survival of histologically confirmed disease within the context of a randomized controlled trial (efficacy). Hypothesis: FV-guided surgery will increase the recurrence-free survival.

2. **Objective 2 (Quality of Life evidence):** To establish the cost per recurrence prevented for this approach and assess quality of life issues. Hypothesis: FV-guided surgery can be delivered in a cost-effective manner and improve the quality of life of patients.

3. **Objective 3 (Scientific/Molecular evidence):** To assess the presence of previously validated molecular markers (microsatellite analysis, LOH) and histological change (quantitative pathology) in surgical margins in a nested case-control study involving a tumor bank created within this project. Hypothesis: FV-guided surgery will spare normal tissue at the same time improving capture of high-risk tissue.
<table>
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| Oral Cancer  
High-grade Precancer | Procedure: Fluorescence visualization device | Phase III |

Study Type: Interventional  
Study Design:  
Allocation: Randomized  
Endpoint Classification: Safety/Efficacy Study  
Intervention Model: Parallel Assignment  
Masking: Double Blind (Subject, Investigator)  
Primary Purpose: Treatment

Official Title: Efficacy of Optically-guided Surgery in the Management of Early-staged Oral Cancer

**Resource links provided by NLM:**
- MedlinePlus related topics: [Cancer](#) [Oral Cancer](#)
- U.S. FDA Resources

**Further study details as provided by University of British Columbia:**

**Primary Outcome Measures:**
- Recurrence-free survival [Time Frame: 5 years] [Designated as safety issue: Yes]

**Secondary Outcome Measures:**
- Histological and molecular evidence of positive margins and quality of life [Time Frame: 5 years] [Designated as safety issue: No]

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<th>Arms</th>
<th>Assigned Interventions</th>
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| A Active Comparator  
All subjects in this study will receive surgery to treat their oral lesions. The margins (or boundaries) of the tissue to be removed during surgery will be defined under regular white light.  
The Control arm: Surgical boundaries for oral lesions will be defined under regular white light.  
Intervention: Procedure: Fluorescence visualization device | Procedure: Fluorescence visualization device  
The trial will randomize 200 patients - 100 in the FV arm (using FV guided the surgery margin) |

| B Experimental  
All subjects in this study will receive surgery to treat their oral lesions. The margins (or boundaries) of the tissue to be removed during surgery will be defined by 2 different procedures (or study arms) in the operating room.  
The FV arm (experimental arm): Surgical boundaries for oral lesions will be defined by FV.  
Intervention: Procedure: Fluorescence visualization device | Procedure: Fluorescence visualization device  
The trial will randomize 200 patients - 100 in the control arm (using conventional white light approach) |
Detailed Description:

1.0. OBJECTIVES AND APPROACHES: 1.1. Objective 1 (Clinical evidence): To assess the effect of FV-guided surgery on the recurrence-free survival of histologically confirmed disease within the context of a randomized controlled trial (efficacy).

Hypothesis: FV-guided surgery will increase the recurrence-free survival. Approaches: This Aim requires the establishment of a randomized controlled trial of 200 patients which will compare outcome for patients in 2 arms: one with conventional surgery with margin delineated under white light, and the other using FV guidance for margin delineation. Please see attached Appendix 1 for a step-by-step protocol. This comprises a multidisciplinary team of surgeons, pathologists, project coordinators, and FV Specialists. In addition to the pre-surgery assessment, all participating patients will have 3-month follow-ups for the first 2 years and 6-month for the rest of the study period. Biopsy will occur when clinically warranted or at 2-year post-surgery.

1.2. Objective 2 (Quality of Life Evidence): To establish the cost per recurrence prevented for this approach and assess quality of life issues.

Hypothesis: FV-guided surgery can be delivered in a cost-effective manner and improve the quality of life of patients.

Approaches: This Aim requires the collection of economic and quality of life (QoL) data to establish the cost per recurrence prevented for FV-guided surgery and to assess quality of life impacts. To assess potential psychosocial consequences of FV-guided surgery, we will measure global QoL. We will use the validated EQ-SD and Functional Assessment of Cancer Therapy Head and Neck Module (FACT-HN) to determine the participant’s QoL at each assessment. The questionnaires will be applied at pre-surgery baseline, and at 6-week, 3-month, and 24-month post-surgery follow-ups.

1.3 Objective 3 (Scientific/Molecular evidence): To assess the presence of previously validated molecular markers (microsatellite analysis, LOH) and histological change (quantitative pathology) in surgical margins in a matched case-control study involving a tumor bank created within this project.

Hypothesis: FV-guided surgery will spare normal tissue at the same time improving capture of high-risk tissue.

Approaches: This Aim requires the retrieval and culling of the archival material for a matched control study. The estimate number of cases reaches outcome is 30 (5% of FV group (100) + 25% of control group (100)). Additionally, 60 matched controls will be selected (matched by gender, age, smoking habit, and anatomical site). This Aim is critical to demonstrate the shift in field, sparing normal tissue while catching high-risk occult tissue. Samples for the nested molecular analysis will be performed in Roslin’s Lab for (microsatellite analysis) and Cancer Imaging at BC Cancer Agency (Dr. Macaulay for qualitative Pathology). The protocols used to analyze these samples have been published.

2.0. STUDY TOOL – VELSCOPE®: We have recently developed a simple hand-held field-of-view device for direct visualization of tissue fluorescence in the oral cavity. This tool is currently commercially available as VELscope® (LED Med Inc., White Rock, BC). We have begun a longitudinal study to explore if FV will improve the surgical margin on outcomes of oral cancer surgery. Between 2004 and 2008, 69 patients with x cm oral cancer entered the study. Each case was treated with surgical resection alone and was followed for at least 12 months. Thirty-eight patients had FV-guided surgery, with the surgical margin placed at 10 mm beyond the perimeter of autofluorescence loss. The remaining patients (control group) had the surgical margin placed at 10 mm beyond the tumor edge defined by standard white-light examination. Among those, 7 of the 60 cases (12%) have developed a recurrence of severe dysplasia, carcinoma in situ or squamous cell carcinoma at the treated site, all in the control group (25% versus 0%, P = 0.002). These data suggest the potential utility of autofluorescence changes within this clinical setting. There is a need to design a larger scale randomized controlled clinical trial to confirm the efficacy of FV-guided surgery.

We are also using FV to monitor the potential re-emergence of regions of autofluorescence loss at treated sites in the cases accrued to the longitudinal study and are currently completing an interim assessment of these monitoring results. Autofluorescence loss persists in some cases, increasing in size and intensity over time and giving rise to a clinical lesion containing dysplasia or cancer.

3.0 Core members of the trial and project management: We have a well-built core group with long-term and strong working relationships, including surgeons (Drs. Anderson (Co-PI) and Duham), Pathologists (Drs. Breken (Co-PI) and Zhang), and Oral Medicine (Drs. Poh (PI) and Williams), and are in a world-leading position in using fluorescence visualization in operating room and in follow-up. Dr. J. Lee, collaborator, from M.D. Anderson Cancer Centre and has extensive experience in clinical trials with special expertise in randomized controlled trials. He will be the trialist for this project, design a program for patient randomization, oversee the trial protocol, and work with local statistician (Prof. Shen) for day-to-day data management. Professor Jihaus Chen, Department of Statistics, the University of British Columbia will serve as the biostatistician to the trial and will be responsible for the data analysis and submission of interim analyses to the Data Monitoring Board.

4.0 Basic trial design: The proposed study will be a double-blinded, randomized controlled Phase III study to evaluate the effect of FV-guided surgery in patients diagnosed with severe dysplasia, carcinoma in situ and invasive squamous cell carcinoma and undergoing surgery treatment with an intent to cure. The trial randomizes 200 patients: 100 in the FV arm (using FV guided the surgery margin) and 100 in the control arm (using conventional white light approach). The trial period is 5 years - 2 years to complete accrual and 3 more years of follow-up.

Eligibility

Ages Eligible for Study: 19 Years and older
Genders Eligible for Study: Both
Accepts Healthy Volunteers: No

Criteria

Inclusion Criteria:
- Patients diagnosed with severe dysplasia, carcinoma in situ, invasive squamous cell carcinoma (T1 or T2) of the oral cavity (CO-O site codes: C02.0-C06.9) who will be undergoing curative resection (primary disease).

Exclusion Criteria:
- Patients with a non-oral malignancy diagnosed (not including non-melanoma skin cancer and lymphoma outside of head and neck region) within the past 3 years.
- Patients with evidence of distant metastasis (as determined by CAT and X-ray) at the time of recruitment.
Contacts and Locations

Please refer to this study by its ClinicalTrials.gov identifier: NCT01039289

Contacts

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