

**A Cross-Sectional Study Evaluating
Chemiluminescence and Autofluorescence in
the Detection of Clinically Innocuous
Precancerous and Cancerous Oral Lesions**

Ravi Mehrotra, Mamta Singh, Shaji Thomas,
Preeti Nair, Shruti Pandya, Niraj Shakti Nigam
and Pankaj Shukla

J Am Dent Assoc 2010;141;151-156

*The following resources related to this article are available online at
jada.ada.org (this information is current as of April 6, 2010):*

Updated information and services including high-resolution figures, can be found
in the online version of this article at:

<http://jada.ada.org/cgi/content/full/141/2/151>

Information about obtaining **reprints** of this article or about permission to reproduce
this article in whole or in part can be found at:

<http://www.ada.org/prof/resources/pubs/jada/permissions.asp>

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

Dr. Mehrotra is a professor, Department of Pathology, Moti Lal Nehru Medical College, Lowther Road, Allahabad 211 001, India, e-mail "rm8509@gmail.com". Address reprint requests to Dr. Mehrotra.

Dr. Singh is a professor, Department of Pathology, Moti Lal Nehru Medical College, Allahabad, India.

Dr. Thomas is a professor, Department of Oral and Maxillofacial Surgery, People's College of Dental Sciences & Research Centre, Bhopal, India.

Dr. Nair is a professor, Department of Oral Medicine and Radiology, People's College of Dental Sciences & Research Centre, Bhopal, India.

Ms. Pandya is a research scholar, Department of Pathology, Moti Lal Nehru Medical College, Allahabad, India.

Dr. Nigam is a consultant dental surgeon, Vidisha, India.

Dr. Shukla is a consultant pediatrician and civil surgeon, District Hospital, Vidisha, India.

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^{4,5} 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.⁶ 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 substantive review of its safety and efficacy.⁶

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.⁷ The 510(k) clearance of VELscope was based on the manufacturer's claim that it, in turn, was "substantially equivalent" to the ViziLite system.⁸

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's⁹ 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 vermilion 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 *t* 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

TABLE

Demographic data for patients.		
VARIABLE	PERCENTAGE OF PATIENTS*	
	ViziLite Plus With TBlue [†] (n = 102)	VELscope (n = 156) [‡]
Median Age (Years)	39	41
Male:Female Ratio	7.5:1	8.7:1
Tobacco Use	73	69
Location of Lesions		
Buccal mucosa	50	54
Retromolar trigone	14	16
Tongue	19	10
Alveolar mucosa	7	9
Gingiva	4	5
Floor of mouth	3	2
Palate	3	4

* 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.

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,¹⁰ Oh and Laskin¹¹ concluded that ViziLite produced reflections that made visualization more difficult than that with typical operatory lighting. In another study, Farah and McCullough¹² 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 colleagues¹³ 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.

Toluidine blue stain. Epstein and colleagues¹⁴ 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."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.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.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,²⁰ clinicians should not rely on VELscope to detect precancerous lesions.

Huff and colleagues²¹ 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 colleagues²² 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.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.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.

The authors are grateful to the Suhsilaben Ramniklal Jhaveri Trust, Mumbai, India, for logistical support for this study.

1. Robinson PN, Mickelson AR. Early diagnosis of oral cavity cancers. *Otolaryngol Clin North Am* 2006;39(2):295-306.

2. American Cancer Society. Estimated new cancer cases and deaths by sex, US, 2008. Available at: "www.cancer.org/downloads/stt/CFF2008Table_pg4.pdf". Accessed Dec. 17, 2009.

3. Mehrotra R, Pandya S, Chaudhary AK, Kumar M, Singh M. Prevalence of oral pre-malignant and malignant lesions at a tertiary level hospital in Allahabad, India. *Asian Pac J Cancer Prev* 2008;9(2):263-265.

4. Linggen MW, Kalmar JR, Karrison T, Speight PM. Critical evaluation of diagnostic aids for the detection of oral cancer. *Oral Oncol* 2008;44(1):10-22.

5. Patton LL, Epstein JB, Kerr AR. Adjunctive techniques for oral

cancer examination and lesion diagnosis: a systematic review of the literature. *JADA* 2008;139(7):896-905.

6. U.S. Department of Health and Human Services. 510(k) clearances. Rockville, Md.: U.S. Department of Health and Human Services, U.S. Food and Drug Administration. "www.fda.gov/cdrh/510khome.html". Accessed Dec. 17, 2009.

7. U.S. Department of Health and Human Services. Indications for use. ViziLite Blue Oral Exam Product. Rockville, Md.: U.S. Department of Health and Human Services, Food and Drug Administration; 2005. "www.accessdata.fda.gov/cdrh_docs/pdf3/K033033.pdf". Accessed Jan. 3, 2010.

8. U.S. Department of Health and Human Services. Section 5-510(k) summary: K060920. Rockville, Md.: U.S. Department of Health and Human Services, Food and Drug Administration; 2006. Available at: "www.accessdata.fda.gov/cdrh_docs/pdf6/K060920.pdf". Accessed Nov. 29, 2009.

9. Sciubba JJ. Improving detection of precancerous and cancerous oral lesions: computer-assisted analysis of the oral brush biopsy. U.S. Collaborative OralCDx Study Group. *JADA* 1999;130(10):1445-1457.

10. Epstein JB, Gorsky M, Lonky S, Silverman S Jr, Epstein JD, Bride M. The efficacy of oral lumenoscopy (ViziLite) in visualizing oral mucosal lesions. *Spec Care Dentist* 2006;26(4):171-174.

11. Oh ES, Laskin DM. Efficacy of the ViziLite system in the identification of oral lesions. *J Oral Maxillofac Surg* 2007;65(3):424-426.

12. Farah CS, McCullough MJ. A pilot case control study on the efficacy of acetic acid wash and chemiluminescent illumination (ViziLite) in the visualisation of oral mucosal white lesions. *Oral Oncol* 2007;43(8):820-824.

13. Kerr AR, Sirois DA, Epstein JB. Clinical evaluation of chemiluminescent lighting: an adjunct for oral mucosal examinations. *J Clin Dent* 2006;17(3):59-63.

14. Epstein JB, Silverman S Jr, Epstein JD, Lonky SA, Bride MA. Analysis of oral lesion biopsies identified and evaluated by visual examination, chemiluminescence and toluidine blue. *Oral Oncol* 2008;44(6):538-544.

15. Zila, a Division of Tolmar. ViziLite Plus with TBlue package insert. Intended use. "www.zila.com/UserFiles/File/ViziliteBlueTBlue630.pdf". Accessed Jan. 3, 2010.

16. Andersson-Engels S, Klinterberg C, Svanberg K, Svanberg S. In vivo fluorescence imaging for tissue diagnostics. *Phys Med Biol* 1997;42(5):815-824.

17. Pavlova I, Williams M, El-Naggar A, Richards-Kortum R, Gillenwater A. Understanding the biological basis of autofluorescence imaging for oral cancer detection: high-resolution fluorescence microscopy in viable tissue. *Clin Cancer Res* 2008;14(8):2396-2404.

18. Chen CT, Chiang HK, Chow SN, et al. Autofluorescence in normal and malignant human oral tissues and in DMBA-induced hamster buccal pouch carcinogenesis. *J Oral Pathol Med* 1998;27(10):470-474.

19. Franzen S, Boxer SG. On the origin of heme absorption band shifts and associated protein structural relaxation in myoglobin following flash photolysis. *J Biol Chem* 1997;272(15):9655-9660.

20. Huat SC, Ping-Ann OV, Hitoshi N, et al. Angiogenic squamous dysplasia in oral epithelial dysplastic lesions. *J Hard Tissue Biol* 2005;14(2):185-186.

21. Huff K, Stark PC, Solomon LW. Sensitivity of direct tissue fluorescence visualization in screening for oral premalignant lesions in general practice. *Gen Dent* 2009;57(1):34-38.

22. Lane PM, Gilhuly T, Whitehead P, et al. Simple device for the direct visualization of oral-cavity tissue fluorescence. *J Biomed Opt* 2006;11(2):024006.

23. Balevi B. Evidence-based decision making: should the general dentist adopt the use of the VELscope for routine screening for oral cancer? *J Can Dent Assoc* 2007;73(7):603-606.

24. LED Dental. Index of /pdf/ guides. "www.velscope.com/pdf/guides/". Accessed Jan. 3, 2010.