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Early Screening and Diagnostic Techniques for Oral Mucosal Lesions

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Objectives

Upon completion of this course, the reader should be able to:

1. Distinguish between adjunctive tools for visual screening and screening tests with tissue sampling.
2. Describe the gold standard for oral cancer screening (the physical oral cancer exam).
3. Discuss the unique characteristics of each visual screening aid.
4. Identify the currently identified risk factors of oral cancer.
5. Understand the basic principles of a surgical biopsy technique.
6. Discuss the differences between an incisional biopsy, and excisional biopsy, and a brush test

Background

Cancers of the oral cavity and oropharynx contribute

to more than 8,500 deaths a year in the United States. Therefore, early detection of potentially premalignant oral mucosal abnormalities is essential to the battle against cancer. Epithelial dysplasia can present as innocuous red, white, or mixed patches on the mucosa in early stages, often mimicking minor soft tissue injury or inflammation. However, ruling out dysplasia is important in the fight against cancer because 12% of dysplasias will become carcinoma in situ within 5 years and 73% of those will progress to metastatic carcinoma.¹ When early diagnosis is made and appropriate intervention and treatment is rendered, the overall survival and patient morbidity is improved.² Although techniques for palpation and incandescent light visual examination have been taught in dental schools for decades, the overall five-year survival rates for oral cancer have only improved about 5% since 1974, fluctuating around 55%.³ Furthermore, in addition to the traditional risk factors of age, race, sex, alcohol and tobacco use⁴, marijuana use⁵, human papilloma viruses⁶, and periodontal disease⁷ have been identified as risk factors. Therefore, several tools and minimally invasive sampling techniques have been introduced for assisting the practitioner in finding mucosal lesions with a high risk of malignant transformation at the earliest possible opportunity. Despite promising case reports about early adjunctive visual screening tools⁸, a lack of understanding about the differences between visual screening tools and tissue sampling techniques combined with early reports of false positive results from cytological sampling have added to the challenge of incorporating early screening technologies in private practice.⁹

Elements of a Physical Oral Cancer Examination

The classical oral cancer screening examination includes visual inspection and manual palpation of the external structures of the head and neck, as well as the internal anatomy of the oral cavity.¹⁰ When possible, bimanual palpation is utilized to identify firm or nodular irregularities, as well as painful trigger points or muscular asymmetry. The overall physical appearance and symmetry should be observed for signs of neurological irregularities.

When examining the external structures of the head and neck, attention should be given to pigmented lesions with raised and irregular borders, non-healing lesions, and scars that may hint to a history of skin cancer therapy. The ears, scalp, and lips are high-risk areas for solar-radiation induced malignancies. (Figures 1 and 2). Lymph nodes should rarely be palpable in health, except in lean individuals. Firm and/or tender nodes should be investigated further for signs of disease or infection. Any suspicious findings should be referred to an appropriately qualified medical provider.



Figure 1. This finding from a clinical examination was diagnosed as squamous cell carcinoma in situ after surgical biopsy.



Figure 2. Despite a negative reported history for skin cancer by this gentleman suffering from dementia, this surgical scar suggests otherwise.

When examining the mouth and oral structures, adequate lighting is critical. Careful attention should be paid to the high risk areas for oral squamous cell carcinoma: retromylohyoid vestibule, tonsillar pillars, anterior floor of the mouth, and lateral borders of the tongue. Any pigmented, white, or red lesions that cannot be easily wiped off should be considered to be suspicious, and a thorough review of the history of the anomaly should be conducted with the patient. Two examples of common benign white lesions might be linea alba and reactive hyperkeratinization. However, jumping to an early conclusion of benign reactive hyperkeratinization without biopsy confirmation should be avoided because squamous cell carcinoma may present similarly in early stages. Erythematous lesions may be benign inflammation secondary to injury, due to post-nasal sinus drainage (Figure 3), or signs of early disease. Raised erythroplakic, leukoplakic, or mixed lesions should immediately be considered suspicious. A reasonable component of the intraoral examination process, if a lesion is discovered is to remove any environmental contributing factor (such as an ill-fitting denture or fractured tooth cusp) and then to reinspect the lesion 14 days later.¹¹ Persistent lesions should be biopsied for definitive diagnosis.

Adjunctive Visual Screening Aids

Two different technologies have been developed to aid in visual inspection of the oral cavity. Chemiluminescence has been developed and initially marketed as Vizilite (Zila Pharmaceuticals, Inc., Phoenix, AZ). A solution of dilute acetic acid is used as a pre-rinse to remove the glycoprotein barrier

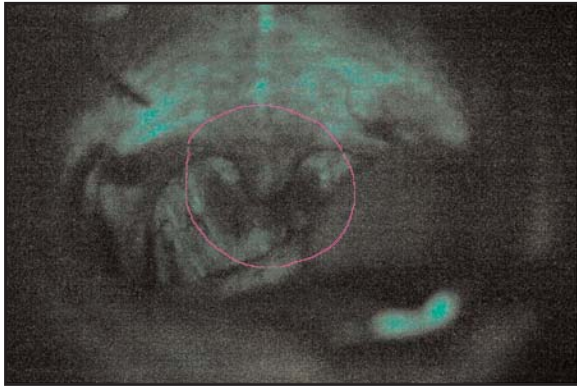


Figure 3. This generalized erythema (outlined), as visualized with the VELscope, with a linear presentation at the back of the oropharynx is consistent with post-nasal drainage and was confirmed with a reported history of exacerbated seasonal allergies.

established by saliva and to dehydrate superficial mucosal cells so that areas of increased nuclear:cytoplasmic ratio can better be visualized. It has been approved for use in patients who are known to be at risk for oral cancer.¹² A proprietary light source is used to "light up" white lesions by reflection and may make red lesions easier to see than by incandescence alone. Other manufacturers have adopted a similar basis for their products (Orascope DK and Microlux DL, both manufactured by AdDent, Inc., Danbury, CT), but they utilize an LED visual light source rather than chemiluminescence. Another technique, developed for use and currently marketed only as the VELscope (LED Dental, Inc., Vancouver, BC) utilizes technology dependent on the natural biofluorescent properties of cellular metabolites¹³ and by the loss of fluorescence associated with the progression of dysplasia that causes breakdown of the collagen matrix.¹⁴ has been cleared for use by the FDA as a safe screening tool in all patient populations as well as for use in surgical margin delineation.¹⁵

Techniques that utilize an acetic acid pre-rinse amplify white lesions and may be used for enhanced visualization of clinically detectable lesions for monitoring and follow-up.¹⁶ Positive findings discovered with the Vizilite Plus may be marked for visualization in incandescent lighting and for photographic documentation with a commercially prepared and stabilized vital dye, TBlue⁶³⁰, which is only available as part of the Vizilite Plus system (Zila Pharmaceuticals, Inc., Phoenix, AZ) for use on



Figure 4. An mixed epithelial lesion was discovered during the conventional oral cancer screening examination. (Photo courtesy of Zila Pharmaceuticals, Inc.)



Figure 5. The same lesion as in Fig. 4 is amplified by chemiluminescence with the Vizilite. (Photo courtesy of Zila Pharmaceuticals, Inc.)

findings previously found during the Vizilite examination. (Figures 4 and 5). Toluidine blue is used for surgical margin identification and for oral cancer research¹⁵, but it is not available in a ready-to-use and convenient form except as TBlue⁶³⁰.

Direct tissue fluorescence visualization is based on the understanding that rapidly reproducing cells do not exhibit the same natural fluorescence as healthy cells. Therefore, it is helpful in identifying erythroplakia, leukoplakia, mixed lesions, and inflammatory lesions. Living cells have natural fluorophores (eg., NADH+, FAD+) that either absorb or generate light when stimulated by an appropriate wavelength of light (ie., blue +/-500nm). The fluorophores in the stroma and epithelium of oral mucosa absorb the blue excitation light and then re-emit light at a longer wavelength (green, yellow or red) a fraction of a second later.



Figure 6. Clinically, there appears to be an area of minor trauma-induced inflammation secondary to irritation from a fractured tooth cusp under incandescent lighting.

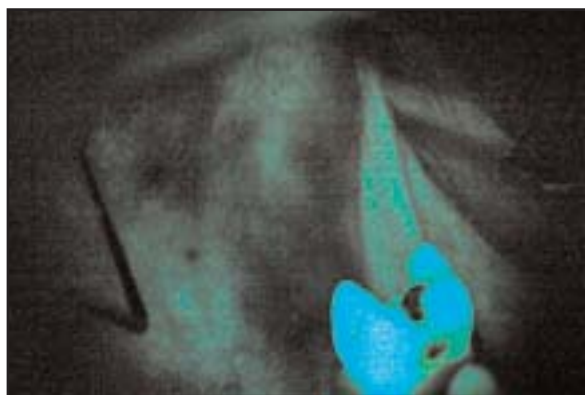


Figure 7. Illumination of the region pictured in Figure 6 with the VELscope reveals a broad dark region extending across the retromolar pad and into the retromylohyoid space, a high-risk zone. This dark area was confirmed by biopsy to be mild epithelial dysplasia.

Human tissue fluorescence is much less bright than the blue light reflected back from the tissue, and filtering is necessary. The VELscope uses a proprietary filtering system in conjunction with a blue light source to enable visualization of the green natural fluorescence of healthy tissues. No additional rinses or chemicals are necessary. Areas of dysplastic stroma appear dark, and healthy tissues appear in variations of green, depending on their collagen substructure. (Figures 6 and 7). A recent retrospective study¹⁷ has shown that the addition of direct tissue fluorescence visualization to the standard physical examination protocol under incandescent light was effective in finding lesions that had not been identified by clinical examination alone and that it was useful in

a private practice setting for detecting potentially premalignant lesions in a stable low-risk population (Table 1).

Minimally Invasive Tissue Sampling

Once a suspicious mucosal lesion is discovered, the decision must be made whether or not a surgical biopsy is indicated. Understanding that only a surgical biopsy will yield a definitive biopsy, some patients may be reluctant to undergo surgical intervention without the presence of obvious pathological signs or symptoms. Furthermore, referral for surgical biopsy of occult lesions discovered with one of the adjunctive visual screening techniques may be questioned by specialists who are unfamiliar with these aids or who question their validity.¹⁸ Therefore, minimally invasive tissue sampling may be desirable. Currently, two prominent systems are available that utilize brushes to collect cells from suspicious lesions, presumably from all of the epithelial layers.

The Oral CDx system (CDx Laboratories, Inc., Suffern, NY, USA) has been heavily marketed. Essentially, cells are collected by the dentist, usually without anesthesia, using a simple rotary technique with a circular brush. The tissue sample is smeared onto a coded glass slide chairside and fixed with a cyanoacrylate solution. The brush and any residual cells are then discarded. The sample slide, once fixed, is shipped to CDx Laboratories. A sophisticated computerized screening system identifies irregular cells. Cytopathologists review the slides and interpret the results. A report is sent to the dentist describing whether the results are "atypical, warranting further investigation", "positive", or "negative for epithelial abnormality."

A similar tissue collection technique using a standard cytology brush is utilized for liquid-based cytology. However, instead of preparing the specimen chairside, the entire brush is placed in a vial containing a proprietary liquid alcohol-based medium. Presumably, this allows testing of the entire sample. The vial containing the brush and cells is then shipped to the laboratory for processing by SurePath protocol (TriPath Imaging, Inc. Burlington, NC, USA). Once centrifuged, slides are processed for modified Papanicolaou staining and examined microscopically by a board-certified oral pathologist. A detailed report is then sent to the submitting clinician with a

recommendation for appropriate follow-up therapy. Both of these tissue tests may be helpful for patient education and in the clinical decision-making process for whether or not to perform a surgical biopsy of a suspicious lesion. However, they are not diagnostic. The results are entirely dependent on the particular site within a suspicious lesion where the brush test is conducted. Any suspicious lesion where the cause cannot be identified by history that persists for more than 14 days should be biopsied surgically for definitive diagnosis.

Surgical Biopsy

Surgical biopsies may be performed with a diode laser or a scalpel. Since marginal tissue may be ablated with peripheral heat generated by a laser, this author prefers the scalpel technique. Regardless of the tool used for performing the biopsy, the technique is similar. There are essentially two types of biopsies: excisional and incisional. Excisional biopsies remove the entire lesion, a border of normal tissue of at least 5 millimeters and a clean connective tissue base.¹⁹ Incisional biopsies include a piece of the lesion, and preferably some healthy border tissue. A tissue punch may also be used for either excision of a small lesion or for incisional biopsies. The technique is similar for any surgical biopsy.

While utilizing brush cytology or performing a surgical biopsy, careful consideration should be given to the type of biopsy chosen and to the location within a lesion to be sampled. Whenever possible, an excisional biopsy is preferable to minimize the risk of seeding a potentially malignant lesion. Incisional biopsies are appropriate for large lesions where complex surgery would be required for total excision. However, whenever an incisional biopsy or brush cytology sample is taken, the clinician must accept that the biopsy is only indicative of the area of the lesion where the sample is taken. Adjunctive visual screening technologies may be helpful in identifying appropriate sampling locations within a large lesion or for surgical margin determination.¹⁵ Obviously, anatomical considerations cannot be ignored so that post-operative morbidity, paresthesia, etc, may be minimized.

After adequate anesthesia, a silk suture is placed directly through the piece of tissue to be removed for biopsy. This is only used for stabilization of the

sample. An elliptical incision is planned and may be marked with pinpoint bleeding stimulation if desired. Marking with ink should be avoided because it complicates histological interpretation of the specimen.¹⁹ Mild tension is applied to the suture. Alternatively, two hemostats may be clipped around the specimen in an end-to-end fashion to stabilize the surgical site (Figure 8). A smooth arced incision is made through the epithelium into the connective tissue, with the blade angled slightly toward the planned secondary incision. A complimentary incision is made to complete the ellipse, angling the blade into the primary incision so that the specimen is cleanly removed. The specimen, with the attached suture if used, is placed into a formalin-based solution and shipped to the lab for histological processing. Once the specimen is removed, the surgical margins are undermined approximately 5 millimeters so that an adequate suture anchor can be achieved. Beginning with the ends of the elliptical surgical opening, interrupted silk sutures are placed approximately 2-3 millimeters apart from each other until primary closure is achieved (Figure 9). Although resorbable sutures may be used, it has been this author's experience that 3-0 or 4-0 silk sutures are easier to place for biopsy closure and encourage compliance with the follow-up appointment for suture removal. Post-operative instructions and appropriate recommendations for management of mild or moderate pain should be given to the patient, and a 1-week follow-up appointment should be scheduled for suture removal and re-evaluation.



Figure 8. Fibroid lesion to be excised is stabilized with hemostats and an elliptical incision is made with two complimentary and communicating longitudinal cuts.



Figure 9. Careful planning of an elliptical biopsy incision permits primary closure to aid in healing and to minimize scar formation.

Discussion

Early mucosal dysplastic lesions can be difficult to identify by visual inspection alone. They may present similarly to inflammation, hyperkeratosis due to trauma, lichenoid lesions, etc., which are benign. Another adjunctive technique has been described called diascopy²⁰ in which pressure is applied to the lesion in question that may be helpful in differentiating inflammation from true pathological tissue changes by blanching of the lesion. However, the only definitive measure of reaching a diagnosis is a surgical biopsy, which may be performed by either laser or scalpel. Cytological sampling techniques such as the OralCDx Brushtest and liquid-based cytology may be helpful in assisting the general dentist and patient in making the decision to perform a biopsy and may be helpful in strengthening the observations made with clinical examination in conjunction with adjunctive visual screening techniques. However, all persistent suspicious lesions that do not disappear within 14 days of observation despite removal of any identifiable local environmental factors or ulcerations that do not heal should be biopsied for definitive diagnosis so that appropriate intervention can be initiated at the earliest possible opportunity. Early detection saves lives.

Years of Study	Oral Ca. Screening Protocol	# of Pts.	# of Brushed Specimens	Brush Analysis Results		Surgical Biopsy Results	
				No Abnormality	Abnormal	Benign	Premalignant
12/1/2005 to 12/1/2006	White Light Exam	959	8	No Abnormality	Abnormal	Benign	Premalignant
				6 of 8	2 of 8	2 of 2	0 of 2
12/1/2006 to 12/1/2007	White Light & Fluorescent	905	12	0 of 12	12 of 12	2 of 12	10 of 12

Table 1. Direct tissue fluorescence visualization with the VELscope led to the discovery of 10 premalignant lesions that had not been discovered the year previously with a conventional examination alone on the same patient population in a general practice.⁶

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EARLY SCREENING AND DIAGNOSTIC SAMPLING TECHNIQUES FOR ORAL MUCOSAL LESIONS

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Questions

EARLY SCREENING AND DIAGNOSTIC SAMPLING TECHNIQUES FOR ORAL MUCOSAL LESIONS

- 1. Oral cancers are responsible for how many deaths per year?**
 - a. 450
 - b. 450,000
 - c. 85
 - d. 8,500
- 2. Of the following, which is not currently an identified risk factor for oral cancer?**
 - a. Human papilloma virus, especially types 16 and 18
 - b. Toothpaste containing real cinnamon
 - c. Chronic periodontitis
 - d. Smoking with alcohol use
- 3. The five-year survival rates for oral cancer have only improved about 5% since the early 1970's. Approximately, what is the current survival rate?**
 - a. 100%
 - b. 25%
 - c. 57%
 - d. 30%
- 4. Gynecologists have been much more successful in battling cervical cancers caused by HPV types 16 and 18 than dentists have with oral cancers most likely because:**
 - a. They are much more proactive in treating early dysplasias.
 - b. They do not have as sophisticated screening tools.
 - c. They are much more conservative and wait for treatment until an obvious lesion is discovered.
 - d. None of the above.
- 5. Studies have shown that as many as 1 in 3 girls age 12 and older have engaged in sexual activity with one or more partners. The highest risk factor for oral cancer in the teen population is:**
 - a. Chewing gum
 - b. Chewing tobacco
 - c. Chronic periodontitis
 - d. HPV
- 6. The extraoral cancer screening examination should include all of the following except:**
 - a. Careful palpation and visual inspection of the vermillion zone of the lips
 - b. Palpation of the scalp
 - c. Palpation of the cervical lymph chain
 - d. None of the above.
- 7. Which of the following are characteristics of suspicious extraoral lesions?**
 - a. Irregular, raised borders
 - b. Unhealing scab-like lesions
 - c. Both of the above
 - d. None of the above
- 8. Generally, palpable lymph nodes that are mobile and soft are not a severe concern because they may be due to common variations in health such as the common cold, flu, etc. However, which of the following best describes a high-risk examination finding?**
 - a. Tender, intramuscular "knot" that gets better with heat
 - b. Firm, sessile, non-tender "ball" just anterior to the sternocleidomastoid muscle
 - c. Caries of the second lower right molar
 - d. None of the above

Questions

EARLY SCREENING AND DIAGNOSTIC SAMPLING TECHNIQUES FOR ORAL MUCOSAL LESIONS

9. Which of the following is considered to be a high-risk location for oral cancer?
- Lateral border of the tongue
 - Anterior floor of the mouth
 - Tonsillar pillar
 - All of the above
10. The tongue should always be distended by grasping it with an unfolded gauze sponge or a glove with stable grip because:
- It is important for the patient to remember that you have conducted an oral cancer examination.
 - The base of the tongue and posterior floor of the mouth is a high risk area, often missed. It has been referred to as "the coffin triangle."
 - Simply asking the patient to stick out their tongue activates the muscles of the floor of the mouth, potentially masking any hidden lesions under the tongue.
 - All of the above
11. What is the distinction between adjunctive visual screening tools and brush testing?
- Testing involves collection of tissue cells.
 - Adjunctive visual screening tools require the collection of tissue cells.
 - None of the above.
 - Both A and B.
12. All of the following adjunctive visual screening aids use an acetic acid pre-rinse except:
- Microlux DL
 - VELscope
 - Vizilite Plus
 - Orascoptic DX
13. The VELscope uses a 10% pre-rinse. The primary limitation of the VELscope is that it can only be used to identify raised leukoplakic lesions.
- The first statement is true; the second statement is false.
 - The first statement is false; the second statement is true.
 - Both statements are true.
 - Both statements are false.
14. The Vizilite Plus system is based on reflection of visible light off white mucosal lesions. There is no need for any type of pre-rinse.
- The first statement is true; the second statement is false.
 - The first statement is false; the second statement is true.
 - Both statements are true.
 - Both statements are false.
15. Which of the following best describes the OralCDx Brushtest?
- The tissue sample is collected with a brush, fixed to a slide chairside, and then screened with computer assistance.
 - The tissue sample is collected with a brush, placed in a liquid-containing vial, and then screened only by pathologists.
 - An acetic acid rinse is applied, and then red lesions are readily identified.
 - Fluorophores are collected and prepared for transfer to the lab after removing them surgically.

Questions

EARLY SCREENING AND DIAGNOSTIC SAMPLING TECHNIQUES FOR ORAL MUCOSAL LESIONS

16. Which of the following is not true about liquid-based cytology?
- Most of the collected cells are transferred to the laboratory for processing.
 - A board-certified oral pathologist reviews every sample.
 - An unarguable diagnosis is readily rendered by an oral pathologist.
 - This technique is not capable of providing a definitive diagnosis.
17. Which media is used for submitting a surgical biopsy sample to the lab?
- Alcohol
 - Saline
 - Formalin
 - Oil
18. Which statement is false about surgical biopsy margins?
- They should always extend 1 Cm beyond the margin of the lesion and be as shallow as possible.
 - They should extend 5mm beyond the lesion margin and extend well into the underlying connective tissue.
 - They should never be on ulcerative tissue.
 - None of the above
19. When conducting a surgical biopsy, a suture may be used to stabilize the sample. What is the most likely reason for sending the suture in the sample to the lab?
- Wicking of the suture may contain important cells.
 - Removal of the unstable suture may destroy the tissue sample.
 - Neither of the above.
 - Both A and B
20. The best type of closure for rapid healing of a biopsy incision is which of the following?
- Secondary
 - Primary
 - Tertiary.
 - None of the above because lasers cauterize the margins and seal the wound.

Clinical Evaluation of a Fluoride Varnish for Cervical Dentin Hypersensitivity

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Objectives

After reading this article, the reader should be able to:

- define dentin hypersensitivity;
- understand the causes of dentin hypersensitivity;
- discuss what is necessary for the proper treatment of dentinal hypersensitivity;
- understand the methods used in this double-blind, randomized, controlled clinical trial; and
- advise patients on available clinical procedures for treating dentin hypersensitivity.

Abstract

The purpose of this study was to compare the degree

of desensitization, as reported by patients on a visual analogue scale (VAS), between a fluoride varnish (Dentsply Professional, York, PA) and the placebo varnish.

Each subject assessed pain when exposed to air and ice. Subjects were divided into "high" ($VAS \geq 50$) and "low" ($VAS \leq 50$) sensitivity groups. The assignment of either active or placebo varnish was done randomly in both groups. Desensitization was measured by VAS change from baseline to 2nd, 8th, and 24th weeks. Comparison between the active and placebo data was analyzed at each of the times using *t* tests ($p \leq 0.05$). In addition, VAS score changes for the active and the placebo groups were compared across times with repeated measures ANOVA (≤ 0.05).

There was clinical relief from dentin hypersensitivity for both active and placebo groups at each period as shown by decreasing VAS scores.

There was no significant difference in relief between the active and placebo varnishes at any time period for either compressed air or Endo Ice applications. When comparing the effectiveness of the active varnish and the placebo across time within pooled groups, the active fluoride group achieved significant relief earlier than the placebo group.

Introduction

Dentin hypersensitivity (DH) is a relatively common, painful dental condition. Typically, the pain is of short and sharp duration and occurs in response to certain stimuli applied to the exposed dentin.^{1,2} They include

stimuli such as tactile, chemical, thermal, or osmotic stimuli. Despite early research of Seltzer et al³ that investigated the relationships between pulp histopathology and clinical signs and symptoms, the relationship between the pulp histopathology and symptomatic teeth exhibiting dentinal hypersensitivity has not been clearly established.⁴

A definition of DH was suggested in 1983⁵, and since has been adopted by an international workshop on the design and conduct of clinical trials for treatment of this condition.⁶ The definition stated: "Dentin hypersensitivity is characterized by short, sharp pain arising from exposed dentin in response to stimuli; typically, thermal, evaporative, tactile, osmotic or chemical, and which cannot be ascribed to any other form of dental defect or pathology."

A number of other conditions are known to present with similar symptoms of dentinal pain. Other causes of the typically short, sharp, dentinal pain include caries, chipped teeth, fractured restorations, some restorative materials, cracked tooth syndrome, and palato-gingival grooves.⁷ Dentinal pain affects more than 40 million people per year in the United States.² Its prevalence, according to Drisko⁸, in the general population was ranged from 3.8% to 57%, whereas, in the periodontal patient population, it was much higher, from 72.5% and 98%.⁸ In a study by Gilliam et al.⁹, its prevalence was between 15% (clinical finding) to 25% (self reported) of the adult population.⁹ Distribution of dentinal hypersensitivity, as reported by Taani et al.¹⁰, was significantly higher among women than men.¹⁰ DH has been reported to peak in the third decade of life and peak again in the fifth decade, especially in periodontal patients.¹¹

Grossman outlined many characteristics for the proper treatment of dentinal hypersensitivity.¹³ The recommendations included: 1) treatment should be non-irritant to the pulp, 2) relatively painless on application, 3) easily performed, 4) rapid in action, 5) effective for a long period and 6) consistently effective without staining effects. There are currently two types of treatment methods that follow the requirements above. They have been extensively studied. One method aims to block the exposed dentinal tubules and the other to reduce the excitability of sensory nerves.

A review of the literature indicated that there was strong evidence for the efficacy of a wide range of

agents for the treatment of DH. However, Addy⁴ cautioned that clinical trials on professionally applied and home-use treatments might have shown a significant improvement in symptoms due to a placebo response and/or regression to the mean. In such studies, professional application of any material to exposed dentin would result in, on average, a 40% or greater improvement, irrespective of the specific treatment.⁴

Fluoride has been used extensively in dentinal hypersensitivity treatments. A recent study showed that the use of fluoride varnish as effective in reducing cervical DH.¹² Clinical studies have demonstrated that 0.4% stannous fluoride, applied twice daily, reduced dentinal hypersensitivity.¹⁴ However, prolonged and consistent use for up to 4 weeks, was necessary to achieve this effect. Sodium fluoride application to dentin leads to formation of calcium fluoride (CaF₂) crystals. The crystal size of CaF₂ is small (about 0.05 um). Other occluding agents such as potassium oxalates, however, produced larger crystals. Since dentinal tubules vary in diameter from 0.9 um at the dentin-enamel junction to 2.5 um at the pulp, smaller crystals of CaF₂ were viewed to be less effective.¹⁴ Studies have shown that NaF reduced dentinal fluid flow from 17 to 25%.¹⁴ Fluoride was also shown to be lost rapidly after application to dentin. Obturation of patient dentinal tubules showed that both fluoride and desensitizing toothpastes (sodium acetate desensitizing toothpaste) removed the smear layer through abrasion and deposited the material onto the dentin surface and into the tubules.¹⁴

A new fluoride varnish was developed which consists of 5% sodium fluoride resin varnish with a fluoride concentration of 22,600 parts per million. It is similar to other products currently marketed. The purpose of this study was to clinically evaluate the degree of desensitization as reported by patients on the visual analogue scale (VAS) between a fluoride (active ingredient) and a placebo resin varnish. The research hypothesis for the study was that the varnish with the active fluoride ingredient would be more effective than the placebo in reducing DH. This would be true when both Endo Ice and compressed air was used on the patient at the 8th, 12th and 24th week. It was also postulated that the active varnish would cause more soft tissue irritation than the placebo varnish at 2, 8 and 24 weeks.

Methods

This study was approved by the Institutional Review Board of the University of Maryland, Baltimore. The study was a double-blind, randomized, controlled clinical trial.

Subjects

All of the subjects were screened using the following inclusion criteria 1) 18-60 years of age with sound, caries-free teeth, 2) stable periodontal condition, and 3) incisors, canines, premolars and molars with exposed cervical dentin that was sensitive to cold and compressed air. A total of 49 subjects were included in the study. The subjects were recruited from the patient pool at the Advanced Education in General Dentistry Clinic at the University of Maryland, Baltimore, and from a private dental practice in north Baltimore. Only one tooth per patient was selected for the study rather than one experimental and one control tooth in each patient to avoid cross-contamination. If the patient had more than one tooth in the quadrant with DH, all of them were treated with the same product but only one tooth was scored for the study. In these cases, the tooth to be evaluated was selected with consideration for accessibility for evaluation and proximity to other sensitive teeth that might interfere with pain assessment. If possible, the most sensitive tooth was selected.

Once accepted into the study, the subject was provided with standard fluoride toothpaste (Pepsodent, Church and Dwight, Princeton, NJ) and a soft toothbrush. The subject was told to use this toothpaste during the 6-week run-in period and during the course of the trial. After the 6-week run-in period, the subject returned for the application of the varnish and was again tested for level of sensitivity.

Procedures

Dental co-investigators were calibrated by the principal investigator following a demonstration of the technique to be used to apply compressed air (Figure 1) and Endo Ice (Figure 2) onto the tooth surface without the use of a timer or ruler.

The responses to the stimuli were recorded by the subject on a standardized visual analogue scale which was 100 mm long ranging from "no pain" to intolerable pain". The clinicians were calibrated by the principal



Figure 1.



Figure 2.

investigator using a set of standardized pictures on the Löe and Sillness's Gingival Index.¹⁵

To secure the subject's response to the stimuli, adjacent teeth were isolated with cotton rolls, wiped with a cotton pellet to remove any debris and maintained in a moist condition until application of the stimuli. At each evaluation, the sensitivity of the tooth to a timed application (2 seconds) of compressed air (from a three-way dental unit syringe at a distance of approximately 2 cm) and a cold stimulus (applying a cotton tip saturated with Green Endo Ice refrigerant spray) (Hygienic Corp., Akron, OH), contacting the surface were recorded by the subject on two different VAS scales. The contact time for the cold test was up to 5 seconds although contact time was not extended beyond necessary to generate a response.

The overall time required to complete the evaluations from the screening appointment to the last appointment was 30 weeks (including the 6 week run-in period).

Randomization

Those reporting with an average VAS greater than or equal to 50 mm were grouped into the "high" sensitivity group while those with an average VAS lower than 50 mm were grouped into the "low" sensitivity group. A random number table was used to assign equal numbers of subjects in each group to either the active or the placebo resin varnish.

Treatment

The application of both varnishes were done according to the manufacturer's recommendation for the active varnish following the initial 6 week standardization period. The active fluoride varnish (Dentsply Professional, York, PA) consists of a resin-based 5% sodium fluoride. This varnish applied using a single-patient delivery system. This system reduces the risk for cross contamination and over-dispensing. The dentin cervical areas then first cleaned with pumice (Figure 3) and water and lightly dried with compressed air. Then the varnish was applied directly from the dispensing syringe to form a uniform, single, thin coat over the exposed tooth area (Figure 4). Subject was instructed to avoid brushing, flossing, eating and drinking for at least 2 hours to avoid mechanical removal of the varnish.



Figure 3.



Figure 4.

Follow Up

The subject returned for 2nd, 8th and 24th week post-treatment follow up. At each time interval, the subject was tested for hypersensitivity, using both Green Endo Ice and compressed air, and for any changes in the soft tissue (using the Loe & Sillness gingival Index).

Statistical Analysis

All the statistical analyses were performed using the software package Statistical Package for the Social Sciences 11.5 (SPSS for Windows, 2001). The sample size of twenty five in each group was determined using the following assumptions: 1) power = 0.80; 2) confidence level = 95% and 3) an expected mean difference of 30 VAS mm along VAS scale from before to after treatment. For each of the time periods, an independent *t* test was utilized to test for a significant difference in sensitization efficacy between the two varnishes. Tissue irritation was measured through use of the Loe & Sillness Gingival Index.¹⁵ For each varnish, Repeated Measures Analysis of Variance (ANOVA) was used for the measurements taken at baseline, 2, 8 and 24 weeks.

Results

A total of 49 subjects participated in the study. There were 11 male and 38 female subjects. The age ranged between 21 and 64 years with a mean age of 41. Only the facial surface of the selected tooth was used. A total of 49 teeth were selected including nine incisors,

13 canines, 21 premolars and six molars. The subjects were divided into 25 high and 24 low sensitivity group members. Twenty-six of those received the placebo while 23 received the fluoride varnish.

Effect of Active Varnish vs. Placebo in the High Sensitivity Group

Endo Ice as Stimulant: At week two, there was no significant difference in average VAS score change between the active and placebo groups ($t = 1.2$, $p = 0.12$). At week eight, there was no significant difference in average VAS score change between the active and placebo groups ($t = 1.54$, $p = 0.07$). At week 24, there was no significant difference in average VAS score change between the active and placebo groups ($t = 0.95$, $p = 0.18$, Table 1).

Cold Air as Stimulant: At week two, there was no significant difference in average VAS score change between the active and placebo groups ($t = 1.2$, $p = 0.12$). At week eight, there was no significant difference in average VAS score change between the active and placebo groups ($t = 0.89$, $p = 0.19$). At week 24, there was no significant difference in average VAS score change between the active and placebo groups ($t = 0.46$, $p = 0.32$, Table 1).

Comparison of the Effect if Active Varnish vs. Placebo in the Low Sensitivity Group

Endo Ice as Stimulant: At week two there was no significant difference in average VAS score change between the active and placebo groups ($t = 0.81$, $p = 0.21$). At week eight, there was no significant difference in average VAS score change between the active and placebo groups ($t = 0.33$, $p = 0.37$). At week 24, there was no significant difference in average VAS score change between the active and placebo groups ($t = 0.76$, $p = 0.23$, Table 2).

Cold Air as Stimulant: At week two, there was no significant difference in average VAS score change between the active and placebo groups ($t = 1.19$, $p = 0.12$). At week eight, there was no significant difference in average VAS score change between the active and placebo groups ($t = 0.83$, $p = 0.2$). At week 24, there was no significant difference in average VAS score change between the active and placebo groups ($t = 0.21$, $p = 0.4$, Table 2).

Comparison of the Effectiveness of the Active Varnish vs. Placebo Varnishes Across Time Within

Pooled Groups

Fluoride Varnish: There was a significant difference between the baseline VAS score and the 8th and 24th week VAS scores. Additionally, there was no significant difference between the 2nd and 24th week VAS scores. No other differences were significant (Table 3).

Placebo Varnish: The baseline and 2nd week VAS scores were significantly higher than the 24th week scores. No other differences were significant. In summary, patients in the active ingredient group reported significant relief from DH by the 8th week but the placebo group did not report significant relief until the 24th week (Tables 3-4).

Discussion

The study evaluated the effect of a fluoride resin varnish and a placebo varnish on dentinal hypersensitivity following a 6 week period of standardization. The evaluation of pain sensation was self-reporting using a visual analog scale (VAS). This method was chosen since it represented the actual patient's sense of discomfort. In order to assess the impact of an experimental medication (fluoride varnish) for reducing DH on patients' expectations and comfort, the use of a VAS was deemed appropriate. However, as VAS is a self-report tool and highly subjective to the patient's psychological, biological status and cultural background, the use of a more objective method of analysis would have been ideal. By providing an objective method of analysis (such as measuring physiological variables and parametric data), it might have helped to replicate the subjective patient evaluation and ensure that the results obtained from the use of the experimental product were consistent and efficient.

It was surprising to find that there was no significant difference between the VAS improvement scores of the active and the placebo groups at any time period. On finding this result, it was decided to run a second analysis across time to determine when significant relief from DH pain occurred within the active and placebo groups when both high and low sensitivity groups were combined.

There were no statistically significant or clinically important differences between the performance of the fluoride varnish and the placebo varnish at any of the

time periods. However, the symptoms of pain or discomfort were totally eliminated in some subjects in the active group by the 8th week. Among some of those who reported improvement, this effect lasted up to 6 months post-operatively. These results indicated that the fluoride varnish was effective in reducing DH by the 8th week while the placebo group did not achieve significant relief until the 24th week (Tables 3-4).

Most subjects in the low sensitivity group reported minor improvements after desensitization from both active and placebo varnishes. The changes in the VAS score were more dramatic within the subjects in the high sensitivity group. The greatest degree of improvement was seen in the high sensitivity group at the 2nd week of cold air application and at the 8th week of Endo Ice application.

Addy⁴ stated that the majority of DH treatments, whether home or office-based, were formulated to occlude the dentinal tubules. It may be that the application of resin-based varnish (with or without fluoride) on exposed dentin was enough to block the patent dentinal tubules. Addy⁴ also cautioned that most clinical trials might be reporting improvement in symptoms due to either, or both, a placebo response and/or regression to the mean.

Drisko⁸ also stated that the evidence for successful clinical trials might be confusing due to the Hawthorne effect. This experimental effect was said to occur fairly frequently and tended to add to the lack of agreement between studies. Studies that have tried to compare the active agents to placebo products in randomized controlled clinical trials aimed at managing DH have reported conflicting results, mainly due to the Hawthorne effect or other study effects.^{4,8,16,17}

Direct comparisons of competitive products were even more difficult to interpret if the study was conducted by the company representing the product. In a more recent study comparing fluoride mouth rinse against a placebo mouth rinse, Yates et al.¹⁸ discussed, at length, the confounding situation due to the Hawthorne effect especially with pain studies. They stated that most of the data for pain produced results favoring the active product but there was no clear evidence for a significant difference between the products. The use of resin varnish as a placebo may have contributed to the confounding factor in this study. The resin varnish

may have actually caused blockage of the patent dentinal tubules. The lack of use of an ideal control with the fluoride treatment, as in Ritter et al.'s recent DH study, may further increase the confounding factor in the study.¹² Further studies should be done in this area to determine the true effect of the resin.

Assuming that the fluoride varnish is effective in treating DH,¹² which does not yet have a product license for use in DH treatment, it may be that the magnitude of the Hawthorne effect overshadowed any therapeutic action. The lack of plaque-related soft tissue changes (as measured by the Løe and Sillness Gingival Index) may also be due to the Hawthorne effect.¹⁸ Oral hygiene may have improved in subjects who knowingly participated in a clinical study simply because of their participation, perhaps as an attempt to please the researcher. This may explain why the Gingival Index score in every subject during the entire study period was zero.

Consistent with other randomized, controlled trials involving DH, the placebo effect was noticed in this study as well. A recent study reported improvement in DH after application of fluoride varnish.¹² However, this study used another fluoride varnish as a control instead of a plain resin varnish. The authors still reported improvement in DH in both groups over time, however, no statistical significance were noted. Both studies, hence showed that active and placebo/control resins were effective. However, the distinction was that the placebo effect in the other study was fluoride whereas in this study it was resin varnish. The placebo effect may equal the treatment effects of known active ingredients such as potassium and fluoride salts. It is the patient's perception that he or she is using an effective agent that triggers the placebo response in subjects.

In the consensus report on dentin hypersensitivity research, some issues regarding pain mechanisms and measurement were discussed. Pain was regarded as more than a mere sensation. It did not always occur in direct proportion to the intensity of a noxious stimulus or the extent of tissue damage. The amount of pain felt was influenced by many factors such as the individual's sex, age, circumstances, present context, previous experiences and current expectations.¹⁹ Personality characteristics also influenced how the individual reacted to noxious stimuli. The emotive reactions differed in acute and chronic pain; the former often caused anxiety while the latter tended to

cause depression. All of these factors could affect pain experience and perception in response to the treatment. DH used to be regarded as a purely peripheral phenomenon, but the role of central factors should not be ignored. Pain thresholds are relatively simple to measure but they are limited in what they convey, and do not provide comprehensive information about the overall nociceptive system that could be revealed by more global rating methods. Pain severity might be assessed by psychophysical rating methods such as VAS, while multiple and interacting components could be revealed by verbal indices such as the McGill pain questionnaire.¹⁹

The effectiveness of treatment for DH should be investigated by randomized, controlled clinical trials. There should be well-established principles and well-designed protocols. Two papers have highlighted the challenges faced by researchers on desensitizing agents.^{18,19} There is a need to establish the "gold standard" for the clinical evaluation of desensitizing agents in order to remove the confounding factors and the Hawthorne effect. Although the VAS has proven to be a useful tool to measure DH pain during the clinical trials, the true nature of DH pain may be more complicated as suggested by Orachardson et al. with regards to the possible role of the central pain pathway involvement in DH.¹⁹ Further investigation into DH pain may help to make recommendations for a future "standard code of research practice" for clinical evaluations of desensitizing preparations.

her assistance in the preparation of this manuscript.

Conclusion

1. The fluoride varnish group reported earlier pain relief than the placebo group.
2. There was no significant difference in efficacy between the fluoride varnish and placebo groups at the 2nd, 8th and 24th weeks.
3. There was no significant difference in soft tissue irritation between the fluoride varnish and placebo groups at the 2nd, 8th and 24th weeks.
4. The effect of the fluoride varnish in reduction of dentinal hypersensitivity may not be fully understood.
5. Dentinal hypersensitivity resolved in both fluoride and placebo groups by the 24th week.
6. Given time, dentinal hypersensitivity may resolve without any intervention.

ACKNOWLEDGEMENT

The authors would like to thank Ms. Peggy Vaccaro for

Stimuli	Week	VAS Score Changes		Active Mean	±S.D.	<i>t</i>	<i>p</i>
		Placebo Mean	±S.D.				
Cold Air	2	1.36	21.08	11	16.48	-1.19	0.12*
Cold Air	8	4.6	24.88	12.3	17.57	-0.83	0.2*
Cold Air	24	17.5	14.75	19.1	21.02	-0.21	0.4*
Endo Ice	2	13.1	22.98	4.4	27.38	0.81	0.21*
Endo Ice	8	22.82	22.71	18.36	37.91	0.33	0.37*
Endo Ice	24	25.5	19.72	17	30.98	0.76	0.23*

Table 1. Results of average VAS score changes between the active and placebo in the HIGH sensitivity groups.

Stimuli	Week	VAS Score Changes		Active Mean	±S.D.	<i>t</i>	<i>p</i>
		Placebo Mean	±S.D.				
Endo Ice	2	5	32.66	19.9	31.63	-1.2	0.12*
Endo Ice	8	13.5	43.11	36.8	35.21	-1.54	0.07*
Endo Ice	24	23	37.79	36.7	37.25	-0.95	0.18*
Cold Air	2	18.3	26.62	31	27.72	-1.2	0.12*
Cold Air	8	24.7	22.14	34.07	31.34	-0.89	0.19*
Cold Air	24	31.8	33.26	37.2	27.21	-0.46	0.32*

Table 2. Results of average VAS score changes between the active and placebo in the LOW sensitivity groups.

	VAS Score Mean	±S.D.
Baseline	73.9 ^{a*}	25.5
Week 2	62.4 ^{a^b}	25.9
Week 8	47.8 ^{b^c}	30.8
Week 24	45.3 ^c	33.4

Table 3.

Comparing the effectiveness of active varnish within the pooled group of high and low sensitivity subjects.

*Groups with the same letter are not significantly different.

	VAS Score Mean	±S.D.
Baseline	67.5 ^{a*}	25.2
Week 2	58.8 ^a	31.0
Week 8	49.8 ^{a^b}	37.0
Week 24	43.4 ^b	29.0

Table 4.

Comparing the effectiveness of placebo varnish within the pooled group of high and low sensitivity subjects.

*Groups with the same letter are not significantly different.

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CLINICAL EVALUATION OF A FLUORIDE VARNISH FOR CERVICAL DENTIN HYPERSENSITIVITY

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Questions

CLINICAL EVALUATION OF A FLUORIDE VARNISH FOR CERVICAL DENTIN HYPERSENSITIVITY

- The fluoride varnish group reported earlier pain relief than the placebo group.**
 - True
 - False
- As reported in clinical studies, what type of fluoride applied twice daily reduces dentinal hypersensitivity?**
 - 0.4% stannous fluoride
 - 1.23% acidulated fluoride
 - Neutral fluoride
 - Fluoride varnish
- Dentinal hypersensitivity resolved in both fluoride and placebo groups by the 8th week.**
 - True
 - False
- Grossman outlined many characteristics for the proper treatment of dentinal hypersensitivity and his recommendations included:**
 - Treatment should be non-irritant to the pulp.
 - Relatively painless on application.
 - Effective for a long period.
 - Consistently effective without staining effects.
 - All of the Above.
- The evaluation of pain sensation in this study was reporting by the investigators using a visual analog scale (VAS).**
 - True
 - False
- Dentin hypersensitivity is characterized by short, sharp pain arising from exposed dentin in response to stimuli; typically, thermal, evaporative, tactile, osmotic or chemical, and which cannot be ascribed to any other form of dental defect or pathology.**
 - True
 - False
- Early research of Seltzer et al³ which investigated the relationships between pulp histopathology and clinical signs and symptoms reported that the relationship between the pulp histopathology and symptomatic teeth exhibiting dentinal hypersensitivity has been clearly established.**
 - True
 - False
- In the consensus report on dentin hypersensitivity research, which of the following issues regarding pain mechanisms and measurement were discussed?**
 - Pain was regarded as more than a mere sensation.
 - Pain did not always occur in direct proportion to the intensity of a noxious stimulus or the extent of tissue damage.
 - The amount of pain felt was influenced by many factors such as the individual's sex, age, circumstances, present context, previous experiences and current expectations.
 - All of the above.
- The effectiveness of treatment for dentin hypersensitivity need not be investigated by randomized, controlled clinical trials.**
 - True
 - False
- Fluoride has been used extensively in dentinal hypersensitivity treatments.**
 - True
 - False
- In this study, dentinal hypersensitivity resolved in both fluoride and placebo groups by the 24th week.**
 - True
 - False

Questions

CLINICAL EVALUATION OF A FLUORIDE VARNISH FOR CERVICAL DENTIN HYPERSENSITIVITY

12. There was no significant difference in efficacy between the fluoride varnish and placebo groups at the 2nd, 8th and 24th weeks.
- A. True
 - B. False
13. Most study subjects in the low sensitivity group reported significant improvements after desensitization from both active and placebo varnishes.
- A. True
 - B. False
14. The amount of pain felt was influenced by many factors such as the present context, previous experiences and current expectations.
- A. Individual's sex
 - B. Individual's age
 - C. Individual's circumstances
 - D. All of the above
15. Addy⁴ stated that the majority of DH treatments, whether home or office-based, were formulated to occlude the dentinal tubules.
- A. True
 - B. False
16. Consistent with other randomized, controlled trials involving dentin hypersensitivity, the placebo effect was not noticed in this study as well.
- A. True
 - B. False
17. The use of resin varnish as a placebo may have contributed to the confounding factor in this study.
- A. True
 - B. False
18. All but one of the following are conclusions of the study.
- A. The fluoride varnish group reported earlier pain relief than the placebo group.
 - B. There was no significant difference in efficacy between the fluoride varnish and placebo groups at the 2nd, 8th and 24th weeks.
 - C. There was no significant difference in soft tissue irritation between the fluoride varnish and placebo groups at the 2nd, 8th and 24th weeks.
 - D. Dental hypersensitivity resolved in both fluoride and placebo groups by the 12th week.
19. The greatest degree of improvement was seen in the high sensitivity group at the 2nd week of cold air application and at the 8th week of Endo Ice application.
- A. True
 - B. False
20. A random number table was used to assign equal numbers of subjects in each group to either the active or the placebo resin varnish.
- A. True
 - B. False

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Vol. 104

**EARLY SCREENING AND DIAGNOSTIC SAMPLING
 TECHNIQUES FOR ORAL MUCOSAL LESIONS**

**CLINICAL EVALUATION OF A FLUORIDE VARNISH
 FOR CERVICAL DENTIN HYPERSENSITIVITY**

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| 12. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
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| 17. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 18. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 19. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 20. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |



In order to evaluate this journal and better meet your needs, please answer the following questions:

- | | Yes | No |
|---|--------------------------|--------------------------|
| 1. Were the course objectives consistent with the course? | <input type="checkbox"/> | <input type="checkbox"/> |
| 2. Were the course subjects of interest and importance to you? | <input type="checkbox"/> | <input type="checkbox"/> |
| 3. Was the material up-to-date, well organized and presented in sufficient depth? | <input type="checkbox"/> | <input type="checkbox"/> |
| 4. Did the instructor demonstrate a comprehensive knowledge of the subject? | <input type="checkbox"/> | <input type="checkbox"/> |
| 5. Do you have any comments or suggestions concerning this course? | | |

6. Are there any subjects you would like to see in future courses?

FIRST FOLD

Place
Stamp
Here

TEST PROCESSING
AMERICAN DENTAL INSTITUTE
4245 Sigler Rd
Warrenton, Va 20187-3940

SECOND FOLD

PLEASE TAPE HERE ONCE AFTER FOLDING