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Improving Oral Cancer Survival: The Role of Dental Providers

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Abstract

Oral cancer accounts for 2 percent to 4 percent of all cancers diagnosed each year in the United States. In contrast to other cancers, the overall U.S. survival rate from oral cancer has not improved during the past 50 years, mostly due to late-stage diagnosis. Several noninvasive oral cancer detection techniques that emerged in the past decade will be discussed, with a brief overview of most common oral cancer chemopreventive agents.

Oral cancer is an important component of the worldwide burden of cancer. Although its incidence ranges around 3 percent of all cancers, relative survival rates are among the lowest of major cancers. An estimated 28,000 new cases of oral cancer were diagnosed in 2007 in the United States despite routine screening exams in current medical and dental practices. Oral cancer is the eighth most-common cancer among white males and the sixth most common cancer among black men in the United States.

Approximately 9,000 deaths occur as a result of these malignancies. More deadly than breast cancer, cervical cancer, and prostate cancer, it has been estimated that oral cancer kills one person, every hour, every day.[1,2]

Several studies suggest that head and neck cancer, particularly tongue cancer, is increasing in young adults both nationally and internationally.[3] Factors that contribute to this rise are still unknown, suspected etiologic agents include smokeless tobacco, various forms of drug abuse, environmental factors, and the human papilloma virus.[4]

The oral cancer survival rate after five years of diagnosis approximates 50-55 percent. In contrast to other cancers (e.g., breast, colorectal, and prostate cancers) the overall U.S. survival rate from oral and pharyngeal cancer has not improved during the past 50 years.[1,2,5] The five-year survival rate is 75 percent for those with localized disease at diagnosis, but only 16 percent for patients with late stages because in the majority of cases, the cancer is diagnosed in stages 3 and 4 with lymph node metastasis.[6,7] Of all oral cancer cases documented by the National Cancer Institute Surveillance, Epidemiology and End Results Program, advanced lesions outnumbered localized lesions more than 2:1.[8]

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Such dismal statistics seem perverse since the disease primarily arises in the surface oral epithelium that is readily accessible to direct visual and tactile examination. The conclusion that at least some lesions are ignored or missed by patients, health care professionals, or both, is inescapable. In part, this may be due to an incomplete understanding or awareness that even small asymptomatic lesions can have significant malignant potential. Health education programs aimed at motivating patients to present earlier have also been largely unsuccessful. A delay in diagnosis and presentation with late-stage disease may be due to patient delay or professional delay, although both may contribute.

The Importance of Early Detection

The prevention of oral cancer and its associated morbidity and mortality hinges upon the early detection of neoplastic lesions, allowing for histologic evaluation and treatment as necessary. Any tool that improves the detection of such lesions should improve the effectiveness of screening methodologies. Although basic oral cancer examination to achieve early detection requires only a 90-second visual and tactile examination, too few practitioners, and dentists in particular, are conducting these exams.[9] Moreover, the identification of high-risk individuals would permit the development and implementation of efficient chemoprevention and molecular targeting strategies.

There is general consensus that clinical stage at the time of diagnosis is the most important predictor of recurrence and death in head and neck cancer patients. The time to diagnosis is influenced by multiple clinical and sociodemographic variables, including patient reluctance to consult a health care professional, due to lack of access that is all too common, especially in patients with low socioeconomic status, SES, as well as professional delay in diagnosing and treating the disease.

Studies have shown that dentists and other health care providers are in desperate need of systemic educational updates in oral cancer prevention and early detection, as they are remiss in the provision of oral examinations and in the detection of early oral cancers.10 Clinicians can increase survival rates if a cancerous lesion is detected at an early stage, or if a precursor lesion (dysplasia) is discovered and treated prior to malignant progression.[11] Recent models determining the value of a populationbased oral cancer screening program show it to be a promising health promotion strategy (especially in high-risk individuals) with significant increases in quality adjusted life years saved, QALY, which await further economic appraisal. 12

The Need to Educate Dental Health Professionals on Early Cancer Detection

The lack of prevention and early detection of oral cancer by health care providers is a worldwide problem. Most dentists claim to perform an oral cancer examination on their patients, but several studies indicate the dentists' lack knowledge in the area of oral cancer etiology and diagnosis.[11]

Despite the wide availability of several written guidelines, no noticeable progress has been made in achieving earlier diagnosis and prognosis of oral cancer in the past decade.[2] In 2000, Horowitz et al., in the conclusion of a nationwide U.S. survey conducted among practicing dentists, stated that there is a need for systematic educational updates in oral cancer prevention and early detection. The need is to reinforce the importance of 1) obtaining complete health histories, including history of risk factors such as tobacco and alcohol consumption; and 2) performing an increased number of oral mucosal examinations. Based on indications by a preponderance of the dentists surveyed, that the emphasis on oral cancer was not comparable to other content areas in their dental schools, the authors also concluded that greater emphasis on oral cancer prevention and early detection should be incorporated into the dental school

curriculum. Dental boards should also include in the clinical portion of their licensure the performance by the applicants of an oral cancer examination.

The American Cancer Society recommends screening for cancers of the head and neck, including oral cancers, every three years in asymptomatic persons between the ages of 20 and 40, and yearly in asymptomatic patients after age 40. Smokers and alcohol users, who are considered high risk, should be examined every year regardless of their age.[13] Dentists need to know that a comprehensive oral cancer examination only takes 90 seconds of their time — a minimal effort, given the resulting benefits to both the patient and the dentist if cancer is detected early.

Visual examination continues to be the gold standard for the detection of early epithelial changes. Criteria for suspicion of an oral leukoplakia or squamous cell carcinoma include changes in surface texture, loss of surface integrity, color, size, contour deviations, or mobility of intraoral or extraoral structures.[14]

Emerging New Clinical Modalities for Early Detection

Recent advancements in oral cancer research have led to the development of potentially useful diagnostic tools at the clinical and molecular level for the early detection of oral cancer. The gold standard for oral cancer diagnosis remains tissue biopsy with a pathologic assessment, but this technique needs a trained health care provider, and is considered invasive, painful, expensive, and time consuming. Recently, scientific research in the field of oral cancer has focused on finding alternative approaches to traditional biopsy, with high expectations in finding a test for oral cancer detection that mimics the Papanicolaou smear, Pap smear, which has significantly improved the early detection and subsequently lowered the mortality rate of cervical cancer.[15]

Recent clinical diagnostic tools developed for the early detection of oral cancer include tolonium chloride or toluidine blue dye, Oral CDx brush biopsy kits, ViziLite, salivary diagnostics, and several imaging devices such as Velscope and multispectral optical imaging systems. To date, none has shown equivalency or been confirmed to be superior to clinical examination.[16,17]

Vital Staining (Toluidine Blue)

Tolonuim chloride also known as Toluidine blue, TB, has been used for decades to aid in the detection of mucosal abnormalities of the cervix and the oral cavity. TB is a metachromatic dye that clinically stains malignant cells but not normal mucosa. Two mechanisms of toluidine blue staining have been proposed. The dye may be taken up by the nuclei of malignant cells manifesting increased DNA synthesis.

Another hypothesis is that the dye can penetrate through randomly arranged tumor cells. The clinical staining procedure involves patients rinsing their entire mouth with the dye, then the physician inspects for areas of blue staining. Malignant lesions stain dark blue; dysplastic lesions stain different shades of blue, depending on the degree of dysplasia.[14] Blue staining in a patient indicates the need for a biopsy. FIGURE 1 depicts a clinical photograph of a positive TB stain (B) and a negative TB stain (D).

Occasionally, a small amount of dye may be retained in normal mucosa. This dye can be wiped away with acetic acid. Surfaces that are rough or keratinous will also retain stain (e.g., the dorsum of the tongue, gingival crevices). Nonmalignant areas of inflammation occasionally stain with toluidine blue; therefore, all positive lesions should be restained in 14 days to decrease the false positive rate. Toluidine blue can also be used to screen patients with previous

carcinoma of the upper aerodigestive tract. These patients are known to be at high risk for a recurrence; therefore, clinicians may add toluidine rinses to their visual examination.[14,18]

From recent studies, a relationship between toluidine blue staining and genetic changes associated with the progression of potentially malignant lesions to oral cancer — such as allelic loss or loss of heterozygosity (LOH) — was demonstrated.[19] Furthermore, the authors demonstrated in a longitudinal study that toluidine blue identified LOH-positive lesions that subsequently progressed to oral cancer.

Chemiluminscence: ViziLite

Chemiluminensce is a noninvasive screening tool targeted at dentists to assist in the identification of suspicious superficial oral lesions. It consists of an acetic acid wash and a single-use “chemi-light stick” that generates a moderately short wavelength light with peak outputs near 430, 540, and 580 nm for illumination of the oral cavity (ViziLite). The use of acetic acid followed by chemiluminescent illumination for visual diagnosis is similar to speculoscopy (an adjunct to Pap smear of the uterine cervix), utilizing the same chemiluminescent light source for cervical diagnosis.[14] Based on the rationale that the visual presentation of cervical and oral/pharyngeal lesions, including SCC, is nearly identical under chemiluminescence, the cervical approach can be adapted to the diagnosis of any cancerous lesions of the oral cavity.

ViziLite is based on the typically greater nuclear content density and mitochondrial matrix of abnormal cells than that of normal cells. The increased nuclear density and the resulting increase in nuclear to cytoplasmic ratio reflect an increase in the proliferative rate and metabolic activity of precancerous cells. After the patient rinses with a dilute acetic acid solution, the dense nuclei of abnormal squamous epithelial tissue will reflect light and appear white when viewed under a diffuse low-energy wavelength light. Normal epithelium will absorb the light and appear dark.[20]

The majority of studies investigating chemiluminescence evaluate subjective perceptions of characteristics of intraoral lesions including brightness, sharpness and texture versus routine clinical examination. As these parameters are highly subjective, it is not surprising that results have been contradictory.16,[18] Recently a combination of both TB and ViziLite systems (ViziLite Plus with TBlue system) has been introduced. FIGURE 2 demonstrates the use of ViziLite after a positive TB stain on the R-lateral border of tongue. The suspicious lesion is seen as a dense white lesion as compared to the adjacent dark normal mucosa. A new chemiluminescence device (MicroLux DL) has also recently been introduced on the market.[14]

Cytology (Oral CDx)

The brush biopsy (CDx) was designed for use on clinical lesions that would otherwise not be subjected to biopsy because the level of suspicion for carcinoma, based upon clinical features, was low. When an abnormal CDx result is reported (atypical or positive), the clinician must follow-up with a scalpel biopsy of the lesion, as brush cytology does not provide a definitive diagnosis.21-23 The intent of the brush biopsy is to obtain cells from a suspicious oral site while avoiding the pain and discomfort of a tissue biopsy, similar to a Pap smear for identifying abnormal cervical cells in routine cervical cancer screening. The designed brush (Oral CDx) is used for epithelial cell collection and samples are eventually fixed onto a glass slide, stained with a modified Papanicolaou test and analyzed microscopically via a computer-based imaging system. Results are reported as “positive” or “atypical” when cellular morphology is highly suspicious for epithelial dysplasia or carcinoma, or when abnormal epithelial changes are of

uncertain diagnostic significance respectively.24 **FIGURES** 3A and 3B illustrate the use of an oral CDx brush on a suspicious buccal mucosa lesion.

Controversy exists over the use of the Oral CDx product because some studies have indicated a high false-positive and a high false-negative rate.[23] There are multiple examples in the literature of studies with essentially opposite findings; therefore, most articles suggest further investigation of the product. A formal biopsy is still indicated if there is clinical suspicion of a lesion regardless of the Oral CDx result.[25-26]

In conclusion, further research with clear objectives, well-defined population cohorts, and sound methodology are required before promoting the extensive use of the brush biopsy or any other diagnostic tool for oral cancer detection.

Imaging Devices

- Photosensitizers
- Spectroscopy and fluorescence
- In vivo microscopy
- Optical coherence tomography

1. PHOTOSENSITIZERS—Topical or systemic application of photosensitizers can selectively render pathologic tissues fluorescent when exposed to specific wavelengths of light, this technique has extensively been used for skin and esophageal cancer.[27,28] This induced fluorescence can be used to identify and delineate areas of pathology. Although the fluorescence may be strong enough to be detected with the naked eye (**FIGURES** 4A-4C), usually some sort of fluorescence detection device is used to enhance fluorescence detection and assist with accurate lesion mapping. Whilst many agents are under investigation, or in clinical use outside of the United States, FDA approval for photosensitizing drugs remains limited. Some promising agents for photodetection include aminolevulinic acid (ALA) (Levulan), hexyl aminolevulinate (Hexvix), methyl aminolevulinate (Metvix), tetra (metahydroxyphenyl) chlorin (mTHPC), as well as porfimer sodium (Photofrin).[29-33]

In a clinical study of 20 patients with oral neoplasms, three hours after the application of topical Photofrin solution, the photosensitized tissues showed a strong red fluorescence, with increasing fluorescence intensity correlating with increasing levels of pathology. Guided by their visible fluorescence, lesions were biopsied at four suspicious sites for each patient. The diagnostic sensitivity using unaided visual fluorescence diagnosis or fluorescence microscopy approximated 93 percent. Diagnostic specificity was 95 percent for visual diagnosis, improving to 97 percent using fluorescence microscopy. The differences between healthy tissue versus dysplasia versus malignancy were all significant ($p<0.05$).[33]

Advantages of a photosensitizer-based diagnostics approach include the capability for 3-D surface and subsurface mapping of lesion margins using available imaging technologies, the ability to inspect large surface areas, noninvasiveness, and the capability for subsequent photodestruction of the photosensitized lesion. Depending on the photosensitizer used and its mode of application (systemic versus topical), imitations include systemic photosensitization over prolonged periods of time, limited penetration depth, the need for specialized fluorescence detection and mapping equipment, and lack of specificity when inflammation or scar tissue are present.

2. SPECTROSCOPY—This term refers to the process of measuring the emission and absorption of different wavelengths (spectra) of visible and nonvisible light. Various types of

optical spectroscopy have been investigated for oral diagnosis. All of these methods have one basic principle in common: the optical spectrum of a tissue contains information about the biochemical composition and/or the structure of the tissue, which conveys diagnostic information. Malignancy-related biochemical and morphologic changes perturb tissue absorption, fluorescence, and scattering properties. The biochemical information can be obtained by measuring absorption/reflectance, fluorescence, or Raman scattering signals.[34] Data is often in the form of graphs, some imaging devices that color code spectral characteristics of tissues also exist, such as the Velscope.

VELSCOPE SYSTEM: For decades the use of tissue autofluorescence has been described to screen and diagnose precancers and early cancer lesions in organs such as the lung, uterine cervix, skin, and, more recently, the oral cavity.[35-37] The concept behind tissue autoflorescence is that changes in the structure (e.g., hyperkeratosis, hyperchromatin, and increased cellular/nuclear pleomorphism) and metabolism (e.g., concentration of flavin adenine dinucleotide [FAD] and nicotinamide adenine dinucleotide [NADH]) of the epithelium, as well as changes of the subepithelial stroma (e.g., composition of collagen matrix and elastin), alter their interaction with light. Specifically, these epithelial and stromal changes can alter the distribution of tissue fluorophores and as a consequence the way they fluoresce after stimulation with intense light (typically, blue light excitation at 400 to 460 nm), a process called autoflorescence. The autoflorescence signal can be directly visualized by the clinician.[35-37]

One of the tissue fluorescence imaging system that have been marketed to dental offices is the Velscope system. In the oral cavity, normal oral mucosa emits a pale green autofluorescence when viewed through the instrument handpiece whilst abnormal tissue displays a decreased autofluorescence and appears darker with respect to the surrounding healthy tissue.[37-38] Studies have shown that Velscope can improve lesions contrast and therefore improve the clinician's ability to distinguish between mucosal lesions and healthy mucosa. Whilst preliminary data on Velscope's specificity and sensitivity are predominantly based on case series and case reports, full-scale clinical trials using different patient populations are needed to establish the diagnostic efficacy of this tool.[18]

Structural and morphological information may be obtained by spectroscopic techniques that assess the elastic-scattering properties of tissue.[39] Pursuant to encouraging preliminary data clinical trials of elastic scattering spectroscopy, sometimes in combination with fluorescence spectroscopy, or imaging, are under way.[34] Other studies combine spectroscopy with polarized light and/or fluorescence imaging, and/or in vivo microscopy. Devices under development and testing include the FastEEM4 System, the Indentafi and the PS2-oral. These clinical studies are still at a relatively early stage, and preliminary results are encouraging.[40-49]

Significant challenges to the use of diagnostic spectroscopy include the often low signal-to-noise ratio, difficulty in identifying the precise source of signals, data quantification issues, and establishing definitive diagnostic milestones and endpoints, especially given the wide range of tissue types contained within the oral cavity. Limited tissue penetration and concerns about mutagenicity when using UV light present further clinical challenges. The abundance of data/information generated in association with our incomplete understanding of the carcinogenesis process tend to render data analysis and interpretation very complex, however, the development of diagnostic algorithms may be able to mitigate this challenge.[39]

3. IN VIVO MICROSCOPR—In vivo confocal or multiphoton imaging resembles histological tissue evaluation, except that 3-D subcellular resolution is achieved noninvasively and without stains. In epithelial structure, resolution of 1 μm has been achieved with a 200–400-μm field of view. Confocal imaging of oral mucosa has resolved subcellular detail in the

lip and tongue and oral squamous cell carcinoma from multiple sites.[50,51] While this technology can provide detailed images of tissue architecture and cellular morphology, a very small field of view and limited penetration depth of 250-500 μm considerably reduce the clinical usefulness of this approach. Multiphoton microscopy resembles confocal, but affords a greater tissue penetration depth, the use of many different wavelengths of light, and less tissue heating[52] (FIGURE 4). Because of high cost and the specialized expertise required to operate such systems, neither approach is clinically feasible in the foreseeable future.

4. OPTICAL COHERENCE TOMOGRAPHY—Optical coherence tomography, OCT, is an optical imaging method first used to visualize human tissue in 1991. It has been since refined and accepted as an imaging modality in ophthalmology. Several systems have been cleared by FDA for such use; one OCT system (Imalux) currently has FDA 510(k) clearance for nonophthalmalogic medical use.

OCT is a high-resolution optical technique that permits noninvasive imaging of surface and subsurface tissues. It has been compared to ultrasound scanning conceptually. Both ultrasound and OCT provide real-time structural imaging, but unlike ultrasound, OCT is based on low-coherence interferometry, using broadband light to provide crosssectional, high-resolution subsurface tissue images (FIGURE 5). With a tissue penetration depth of 1 mm to 2 mm, the imaging range of OCT technology is suitable for the oral mucosa.[53-56] Previous studies using OCT have demonstrated the ability to evaluate macroscopic characteristics of epithelial, subepithelial, and basement membrane structures and show the potential for near histopathological-level resolution and close correlation with histologic appearance.[57]

In one of the authors' recent studies of 50 patients with oral dysplastic and malignant lesions, intraand interobserver agreement between diagnoses based on histopathology and imaging data was excellent, with kappa values of 0.844-0.896.[58] For detecting carcinoma in situ or squamous cell carcinoma, SCC, versus noncancer, sensitivity and specificity were 0.931; for detecting SCC versus all other pathologies, sensitivity was 0.931 and specificity was 0.973. These data demonstrate the excellent capability of in vivo OCT for screening high-risk patients, monitoring existing lesions, and detecting, diagnosing oral premalignancy and malignancy in human subjects. This study showed that the in vivo OCT image of a dysplastic lesion (FIGURE 6B) parallels histolopathological status (FIGURE 6C), showing epithelial thickening, loss of stratification in lower epithelial strata, epithelial downgrowth, and loss of epithelial stratification as compared to healthy oral mucosa (FIGURE 6D).

For oral cancer, FIGURES 7A AND 7C show clinical appearance and histopathology, respectively of an area of SCC on the buccal mucosa. In the OCT image (FIGURE 7B), the epithelium is highly variable in thickness, with areas of erosion and extensive downgrowth and invasion into the subepithelial layers. The basement membrane is not visible as a coherent landmark.[58]

As the technology and techniques evolve, this modality should progressively reduce the need for biopsy, define surgical margins, and provide a direct evaluation of the effectiveness of complete lesion removal.

Saliva as a Diagnostic Tool

Salivary diagnostics has come to the forefront of biomedical research. The ability to use salivary biomarkers (i.e., DNA, RNA, and proteins) as a predictive measure for systemic disease has generated much interest among dental researchers in the United States and Europe. Laboratory-based methodologies, which allow the rapid identification of proteins, RNA and DNA have afforded scientists the ability to examine and quantify complex salivary profiles. At the University of California, Los Angeles, School of Dentistry, Dr. David Wong and

collaborators are developing research platforms toward the global identification of disease signatures in saliva.

The premise of their approach is that serum contents, such as disease biomarkers, are largely present in saliva, thus rendering oral fluid a logical source to harness disease biomarkers.[59] They employ both a proteome-wide as well as a genome-wide approach toward the identification of disease biomarkers and signatures. Dr. Wong's goal is to develop and utilize novel patient-oriented genomewide molecular tools that may identify oral cancer specific molecular markers.

Early work by Wong and his coworkers identified interleukin 6 and 8 as predictive biomarkers for oral cancer.60-[63] They are now validating these findings and have expanded this work to breast and pancreatic cancer. Oral fluid–based tests presently exist or are being developed to detect a variety of infectious diseases (including HIV, parvovirus, acute hepatitis, dengue fever, and malaria), as well as to detect alcohol and drug use and steroid hormone levels.[64]

Other body fluids such as serum could possibly also be helpful in identifying common genetic mutations, promoter hypermethylation, and LOH.[65,66]

The UCLA group is at the final stage of developing an oral fluid nanosensor test device that could be used in the dental office. This portable, point-of-care, chairside device is to be used for saliva diagnostics, not only for oral cancer but other diseases such as diabetes, Sjögren's syndrome, and breast and prostate cancer (FIGURE 8).

Collectively, technology platform advancement and the identification and validation of robust and discriminatory suites of salivary biomarkers for disease diagnostics represent the necessary marriage to propel saliva diagnostics into a clinical and commercial reality. At the same time, they are building the scientific foundation toward the use of saliva as a diagnostic fluid. This is a perfect example of translational research in reverse, based on a highly relevant clinical observation that saliva contains proteomic and genomic biomarkers for oral cancer detection, and building a scientific foundation toward the mechanistic background, thereby enabling better exploitation of the full clinical potential of saliva diagnostics.[67-69]

Oral Cancer Chemoprevention

Many epidemiologic studies have consistently linked the abundant consumption of foods of plant origin, such as fruit, vegetables, whole grains, legumes, nuts, seeds, and tea, with a decreased risk of developing various types of cancers.[70,71] Chemoprevention is the use of pharmacologic or natural agents that inhibit the development of invasive cancer. These work either by blocking the DNA damage that initiates carcinogenesis, or by arresting or reversing the progression of premalignant cells in which such damage has already occurred.

Recent advances in understanding the causes of cancer and the consequent ability to provide a genetic diagnosis of susceptibility necessitate the identification of agents that can effectively reverse, halt, or at least delay the carcinogenic process. It is important that any agents selected on the basis of trials in premalignant lesions have minimal or no toxicity since a large number of subjects whose lesions are unlikely to progress to cancer will necessarily be exposed to the product. Therefore, the development of new agents or the use of old agents at nontoxic doses is important.[72]

As in all cancers, the most effective way of approaching oral cancer prevention is to identify individuals who are at a high risk to develop such cancers (e.g., individuals with oral premalignant lesions) and to treat them with agents that can suppress the development of additional premalignant lesions and inhibit the development of oral cancer in existing lesions.

Oral carcinogenesis is a multistep process, which is characterized by genetic, epigenetic, and phenotypic changes. Many of these changes involve the activation of signaling or metabolic pathways that give the cells favorable growth and survival characteristics. Therefore, chemopreventive agents that can inhibit or reverse these changes by targeting specific molecular pathways have received increased attention as novel candidates for cancer prevention and therapy.[73]

A wide range of compounds has been investigated as possible chemopreventive therapies for potential oral cancer, such as vitamins and minerals, including vitamin A and other retinoids, beta carotene, vitamin E, vitamin C, folates, and selenium, and has been studied. Herbal treatments have generated interest recently. Molecularly targeted approaches have included cyclooxygenase-2 (COX-2) inhibitors, EGFR inhibitors, and adenovirus vectors.[74] Several promising new compounds are currently in clinical chemoprevention trials in head and neck cancer. These include curcumin analogs, green tea extracts (GTEs), selenium, polyphenols of pomegranate juice, Bowman-Birk inhibitor (BBI) from soybeans and others.75

The authors and other collaborators from multiple institutes are involved in a multicentric phase II NCI-sponsored study to examine the effect of the protease inhibitor Bowman-Birk Inhibitor concentrate (BBIC), on oral leukoplakia patients (**FIGURES** 9A and 9B). This compound had previously been tested in patients with benign prostatic hypertrophy, ulcerative colitis, and those studies suggested clinical activity without toxicity being noted.[76] BBIC effect in the oral cavity is still not well established, but preliminary results are promising.[77]

Because of their easy accessibility to topical treatment and visual clinical examination, oral premalignant lesions provide an excellent model to study the effects of various chemopreventive agents on epithelial solid tumors. In case of oral premalignancy, the primary (clinical) endpoints are determined by clinical measurement of the lesion and confirmed by histologic examination of oral biopsy. Both are feasible in the oral cavity. Secondary (biomarkers) endpoints are usually biochemical and molecular markers found on tissues or buccal cells which are easily obtained from the oral cavity.

Current clinical trials that use clinical and biomarker endpoints will identify useful agents for oral cancer prevention and provide a better understanding of the carcinogenesis process by investigating the specific targeted cellular proteins and dependent cell signaling pathways modulated by these agents. Such insights will not only shed a light on the mechanism of cancer progression but will also guide future drug development and ultimately improve therapy.[78]

THE PREVENTION of oral cancer and its associated morbidity and mortality hinges upon the early detection of neoplastic lesions.

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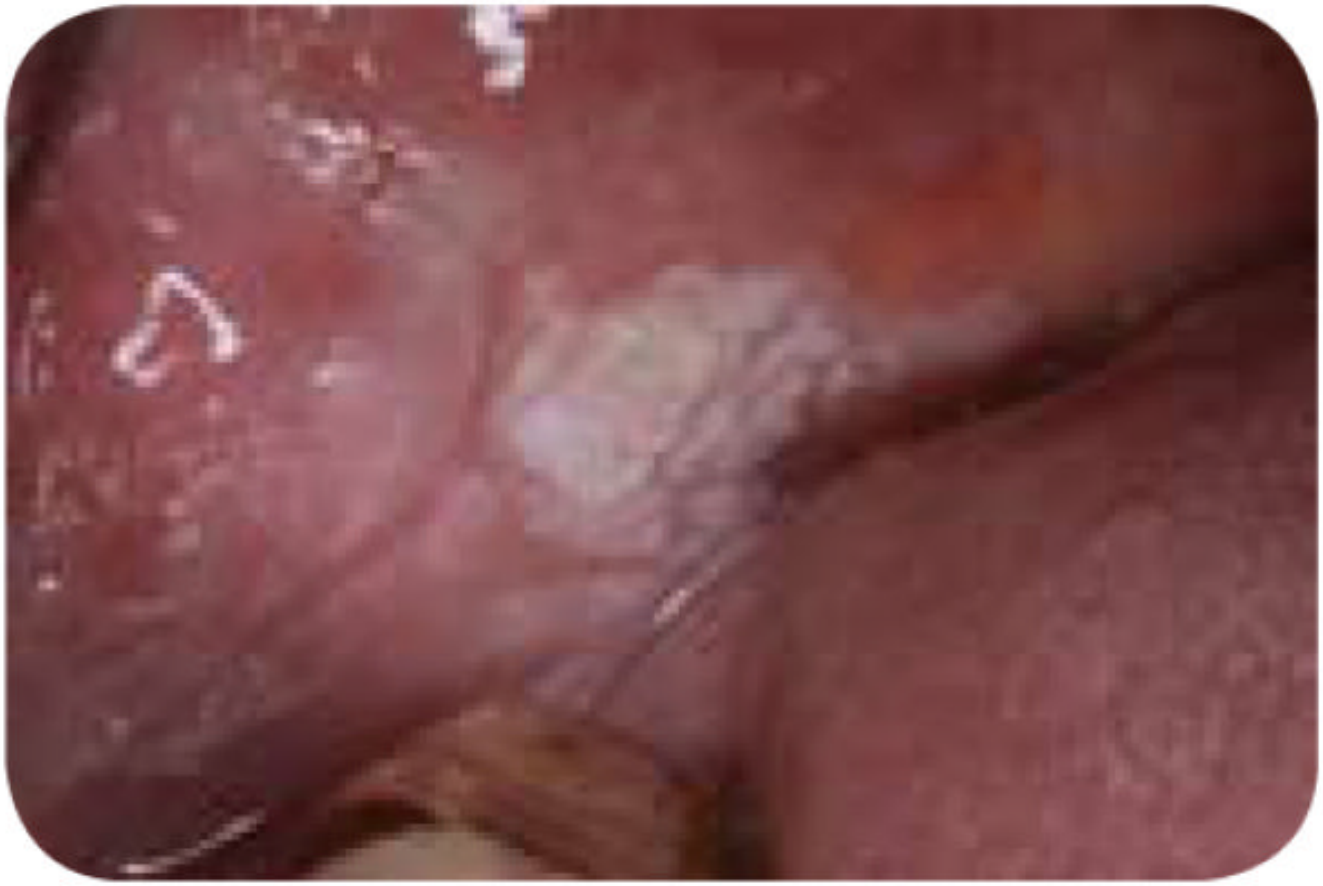

FIGURE 1A.
Leukoplakia lesion on right buccal mucosa.

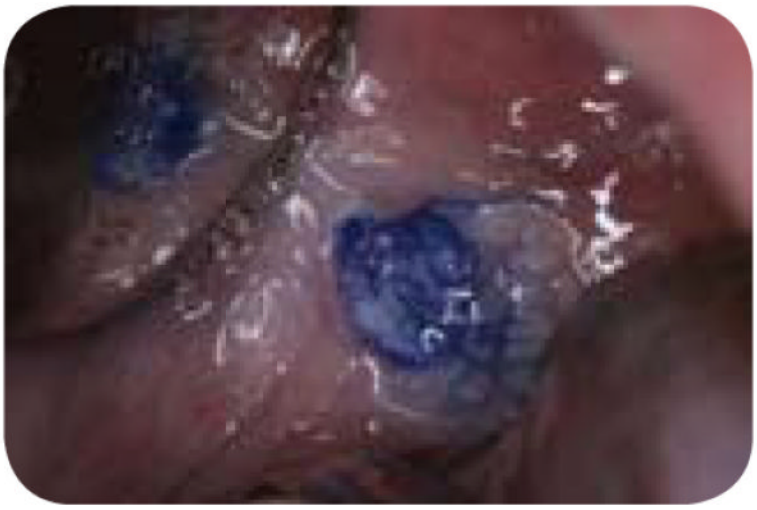

FIGURE 1B.
Positive blue staining after TB rinse.

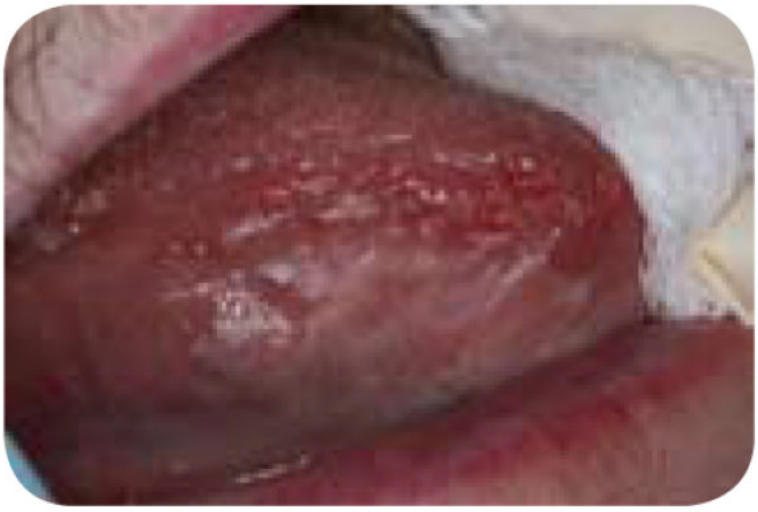

FIGURE 1C.
Leukoplakia of R lateral border of tongue.

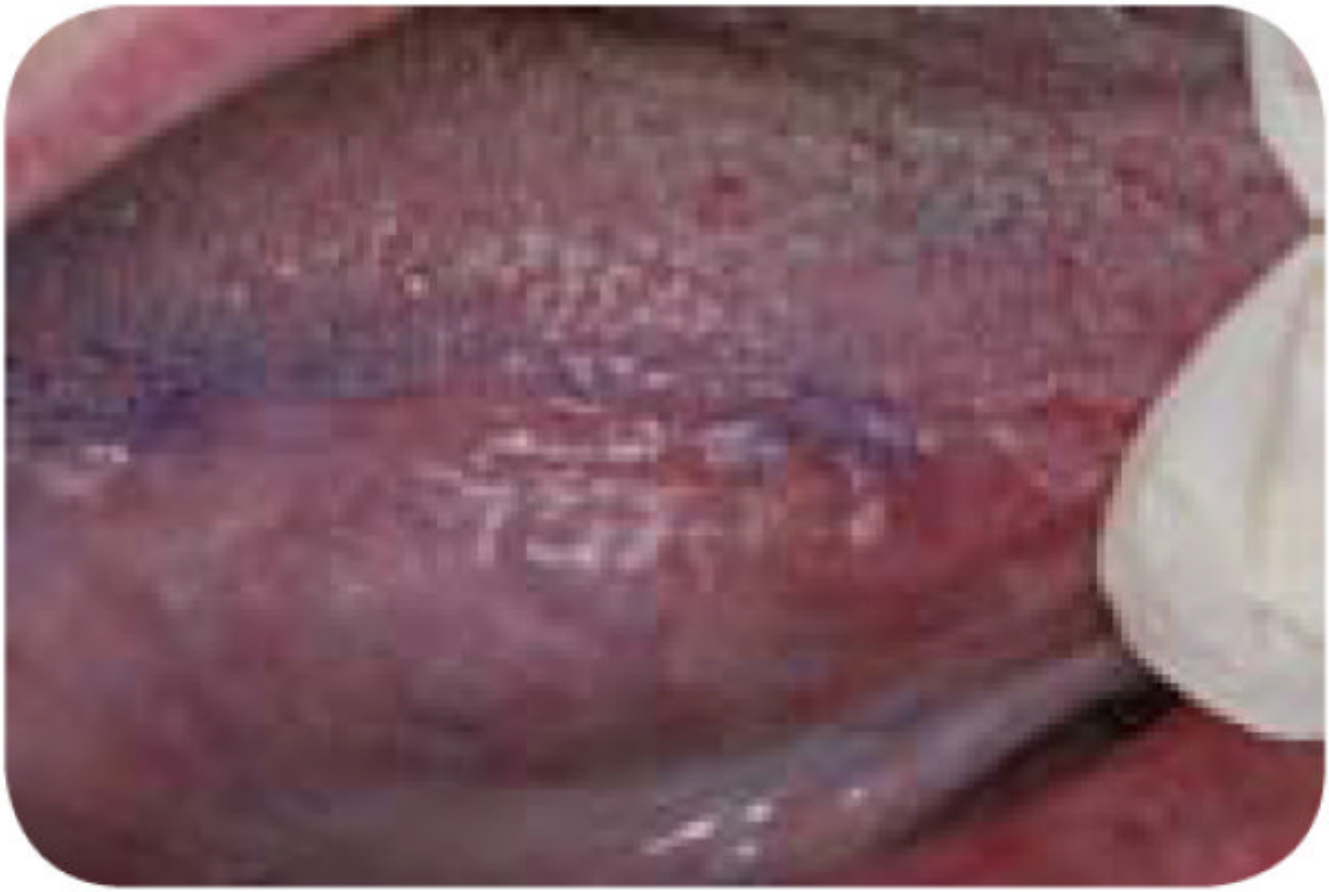

FIGURE 1D.
Negative stain after TB rinse.

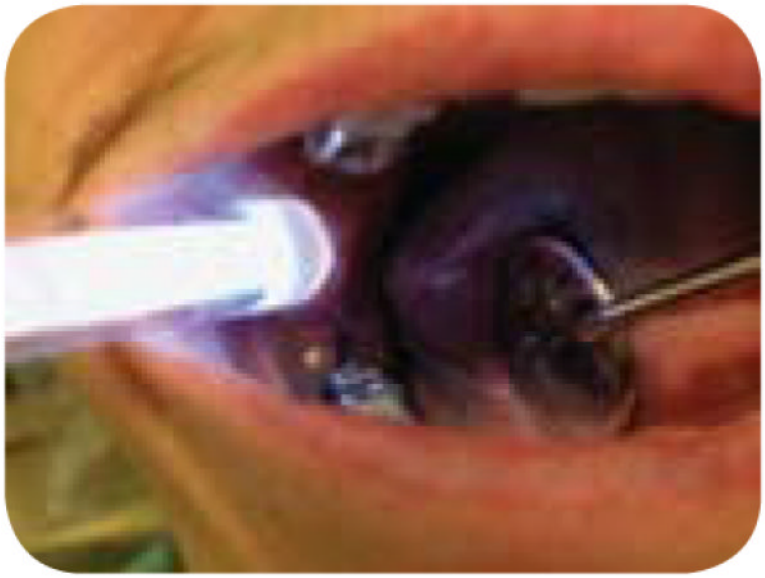

FIGURE 2.
Chemiluminescence with ViziLite shows suspicious lesion on the right side of the tongue as white; normal epithelium is dark.

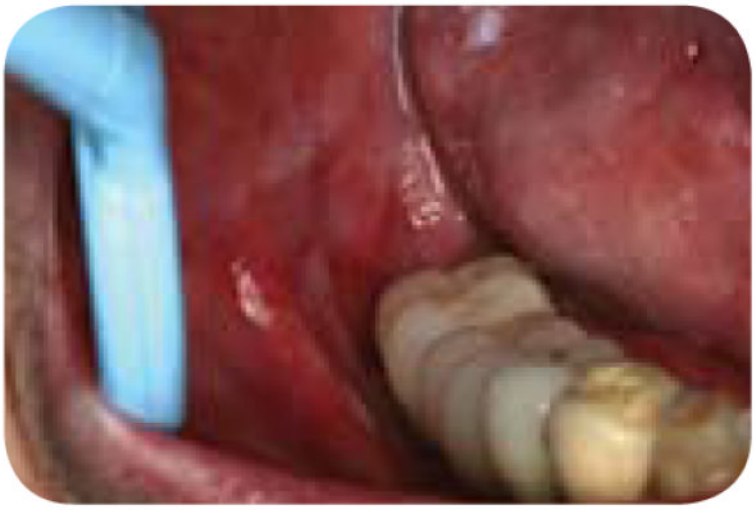

FIGURE 3A.
Oral CDx brush application on a suspicious buccal mucosal lesion, with suspicious erythroplkia lesion on R buccal mucosa.

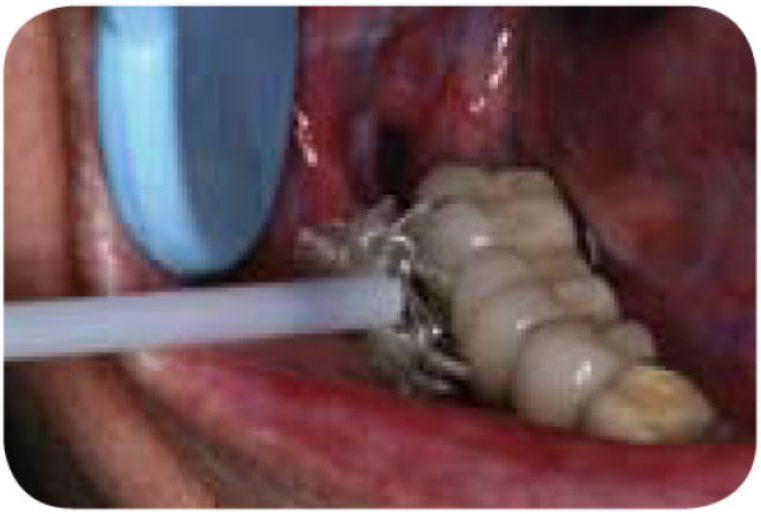

FIGURE 3B.
Application of the Oral CDx brush for cytology testing.

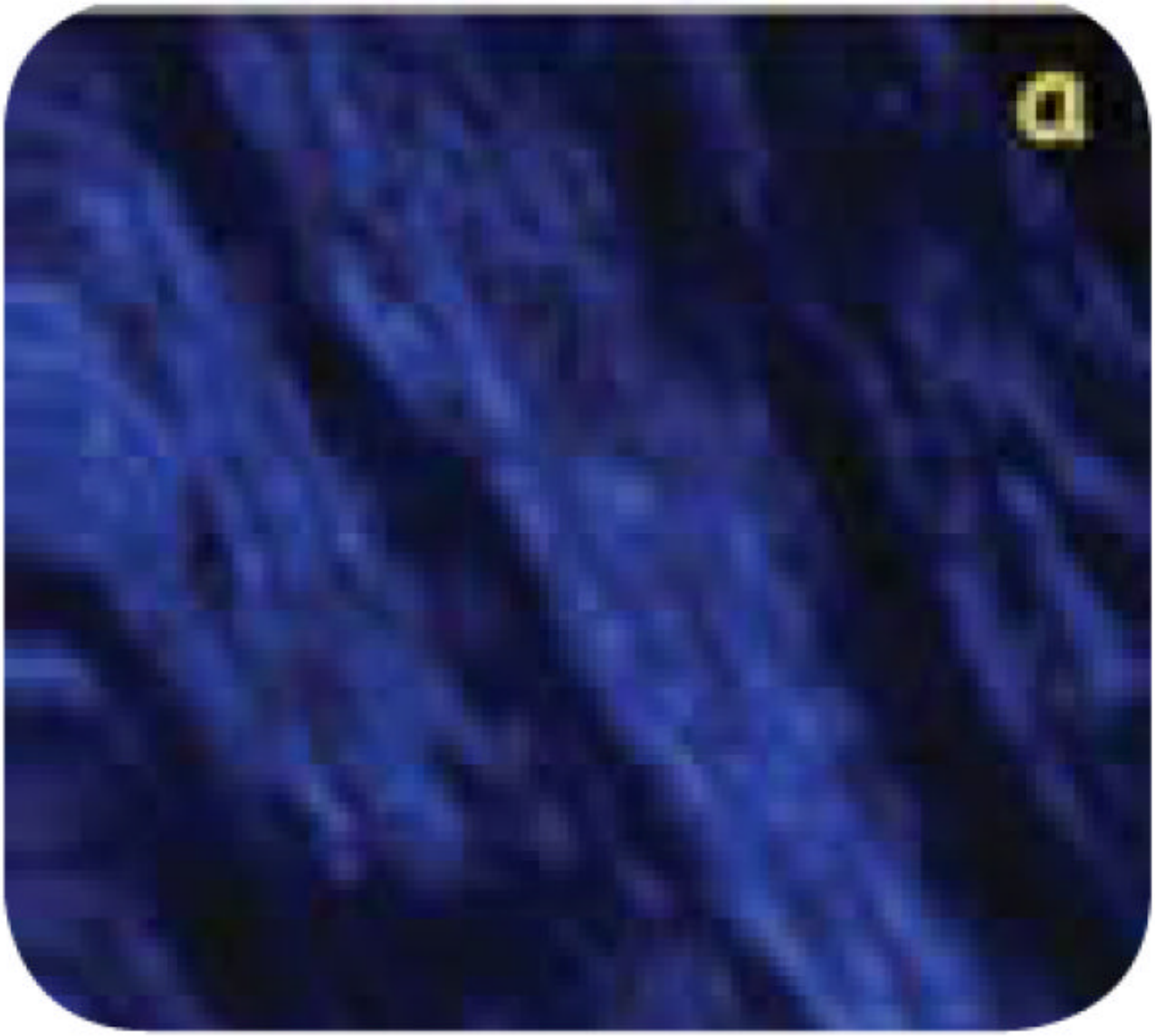

FIGURE 4A.
In vivo multiphoton microscopy images of the hamster cheek pouch. Healthy tissue, showing ordered and dense collagen fiber network in blue.

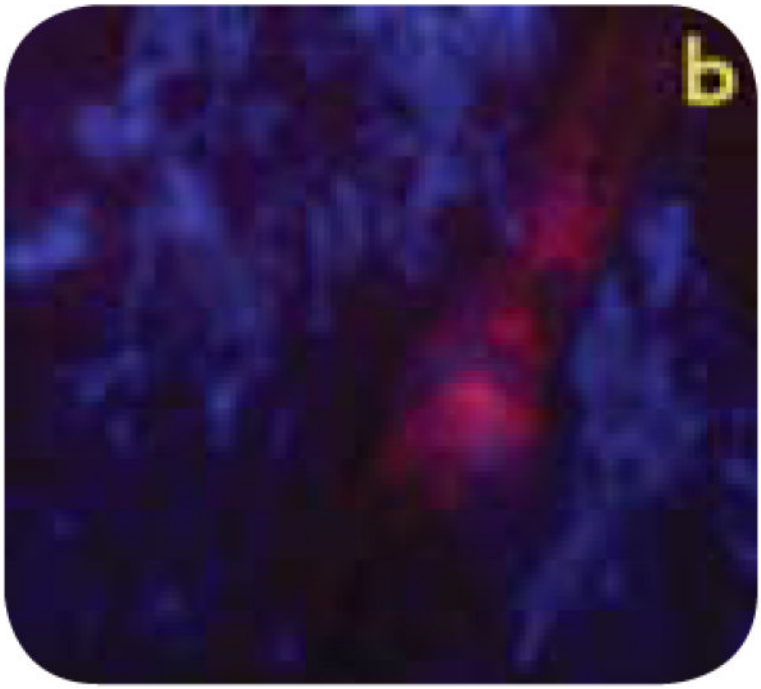

FIGURE 4B.
Dysplastic lesion, showing engorged blood vessel, shortening of collagen fibers with reduced density and organization.

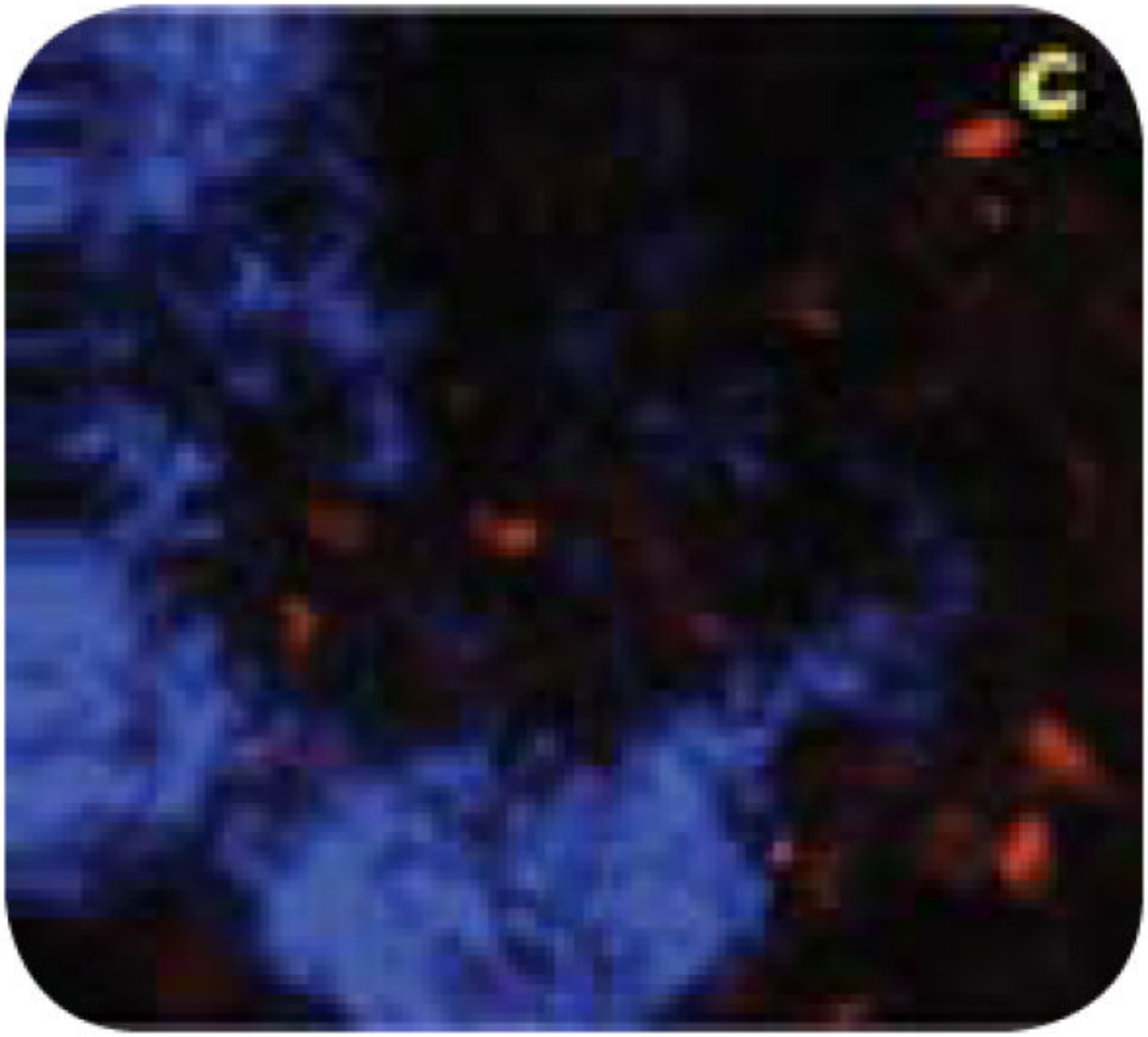

FIGURE 4C.
Malignant lesion, showing further collagen loss and inflammatory exudates (red).

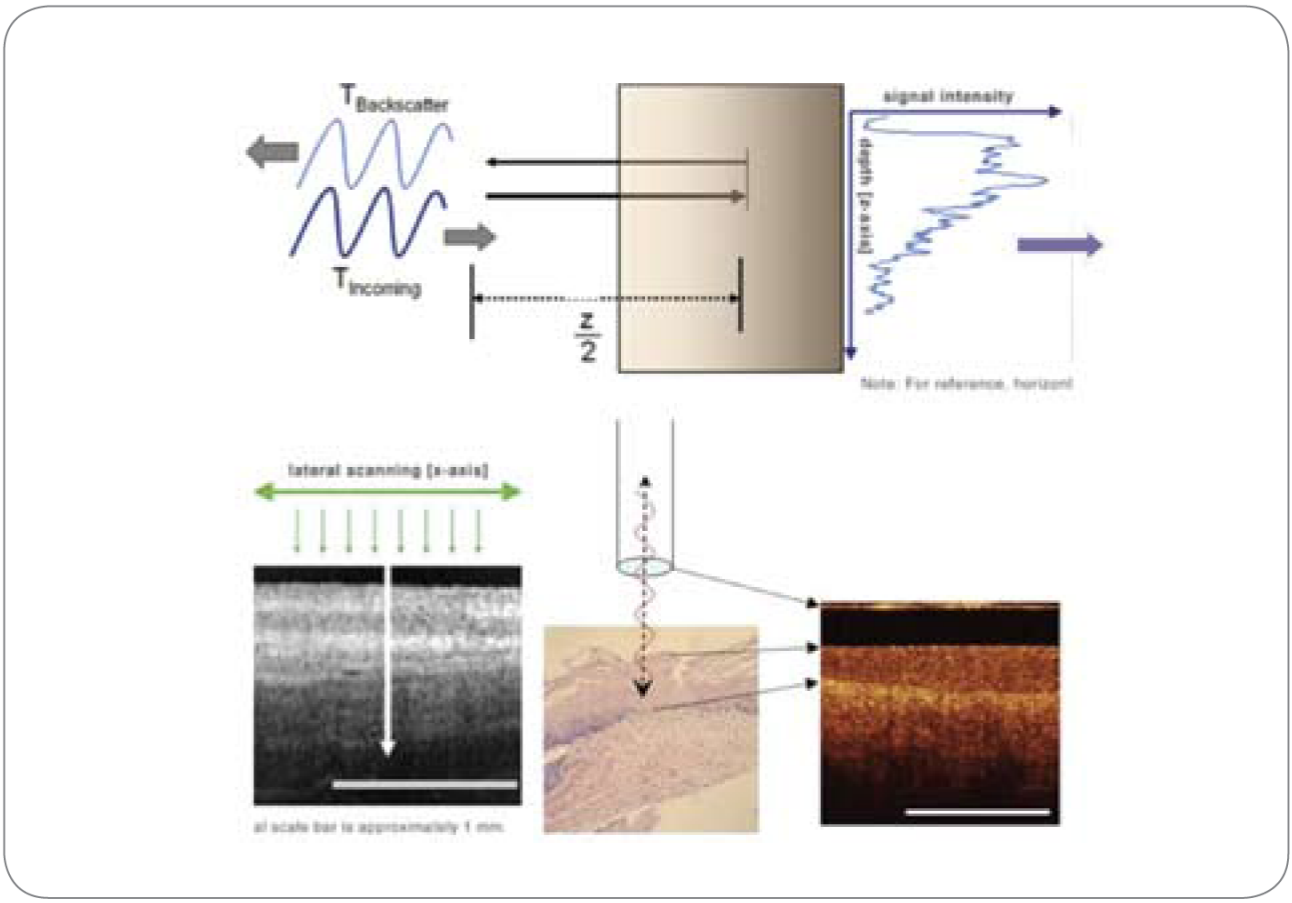

FIGURE 5.

OCT performs 2-D imaging in biological tissues by projecting harmless near-infrared light onto the tissue and measuring the backscattered intensity of light as a function of depth (left). It is able to capture tissue substructure images in real-time using lateral scanning (middle). In-depth spatial resolution is 5-20 μm in air, and is typically able to visualize tissue to a depth of 2 mm (right).

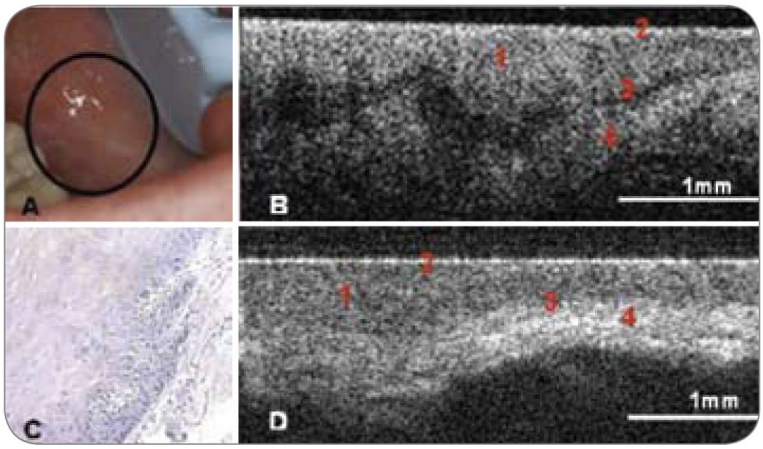

FIGURE 6.

Dysplastic and normal buccal mucosa. **(A)** Photograph, **(B)** in vivo OCT image, and **(C)** H&E (10x) of dysplastic buccal mucosa. **(D)** In vivo OCT image of normal buccal mucosa. Key: 1-stratified squamous epithelium, 2-keratinized epithelial surface layer, 3-basement membrane 4-submucosa.

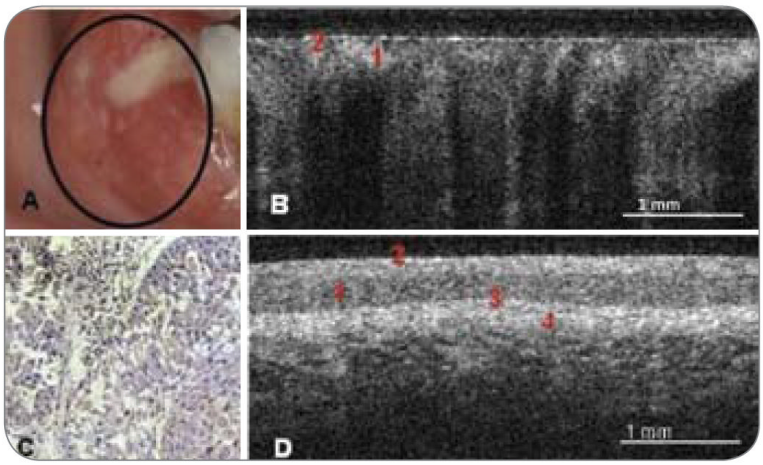

FIGURE 7.

Squamous cell carcinoma of the buccal mucosa. **(A)** Photograph, **(B)** in vivo OCT image and **(C)** H&E (10x) of buccal mucosa with squamous cell carcinoma. **(D)** In vivo OCT image of normal buccal mucosa. Key: 1-stratified squamous epithelium, 2-keratinized epithelial surface layer, 3-basement membrane, 4-submucosa.

FIGURE 8.
The UCLA oral fluid nansosensor test (OFNASET): Showing two chips, one for oral cancer screening and one for Sjögren syndrome screening (*courtesy of Dr. David Wong*).

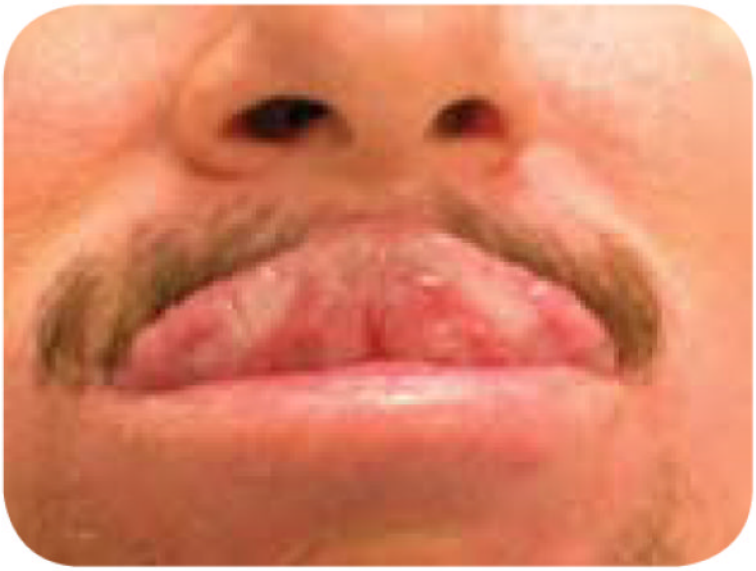

FIGURE 9A.
A patient with proliferating verrucous leukolplkia (PVL) of ventral surface of tongue.

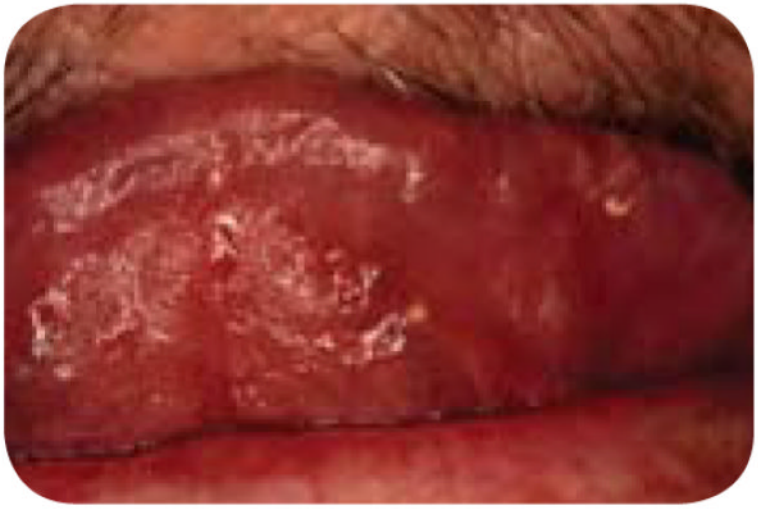

FIGURE 9B.
Regression of the PVL lesion after six months of BBIC treatment.