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TRIOLOGICAL SOCIETY CANDIDATE THESIS

The Color of Cancer: Margin Guidance for Oral Cancer Resection Using Elastic Scattering Spectroscopy

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Objectives/Hypothesis: To evaluate the usefulness of elastic scattering spectroscopy (ESS) as a diagnostic adjunct to frozen section analysis in patients with diagnosed squamous cell carcinoma of the oral cavity.

Study Design: Prospective analytic study.

Methods: Subjects for this single institution, institutional review board–approved study were recruited from among patients undergoing surgical resection for squamous cell cancer of the oral cavity. A portable ESS device with a contact fiber-optic probe was used to obtain spectral signals. Four to 10 spectral readings were obtained on each subject from various sites including gross tumor and normal-appearing mucosa in the surgical margin. Each reading was correlated with the histopathologic findings of biopsies taken from the exact location of the spectral readings. A diagnostic algorithm based on multidimensional pattern recognition/machine learning was developed. Sensitivity and specificity, error rate, and area under the curve were used as performance metrics for tests involving classification between disease and nondisease classes.

Results: Thirty-four (34) subjects were enrolled in the study. One hundred seventy-six spectral data point/biopsy specimen pairs were available for analysis. ESS distinguished normal from abnormal tissue, with a sensitivity ranging from 84% to 100% and specificity ranging from 71% to 89%, depending on how the cutoff between normal and abnormal tissue was defined (i.e., mild, moderate, or severe dysplasia). There were statistically significant differences in malignancy scores between histologically normal tissue and invasive cancer and between noninflamed tissue and inflamed tissue.

Conclusions: This is the first study to evaluate the effectiveness of ESS in guiding mucosal resection margins in oral cavity cancer. ESS provides fast, real-time assessment of tissue without the need for pathology expertise. ESS appears to be effective in distinguishing between normal mucosa and invasive cancer and between "normal" tissue (histologically normal and mild dysplasia) and "abnormal" tissue (severe dysplasia and carcinoma in situ) that might require further margin resection. Further studies, however, are needed with a larger sample size to validate these findings and to determine the effectiveness of ESS in distinguishing visibly and histologically normal tissue from visibly normal but histologically abnormal tissue.

Key Words: Oral cancer, scattering spectroscopy, optical biopsy, noninvasive diagnosis.

Level of Evidence: NA

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INTRODUCTION

Oral cancer constitutes a significant global health problem with over 300,000 new cases and over 145,000 deaths worldwide in 2012.[1] It is estimated that 39,500 to 45,780 new cases of oral cavity and oropharyngeal cancer will be diagnosed in the United States and an estimated 7,500 to 8,650 people will die from these types of cancer in 2015.[2,3] Globally, oral cancer remains a lethal disease for nearly half of all patients diagnosed annually, in part because most cases are already in advanced stages at the time of detection and because local recurrence following treatment is common.

Management of oral cavity cancer may include surgical excision, radiation therapy, or both. Early stage, superficial cancers (stages I and II) can be treated with surgery alone or radiation therapy alone, but surgery is often preferred.[4] Advanced cancers (stages III and IV) are typically treated with surgery and radiation therapy, with or without chemotherapy, depending on the stage of the disease at the time of diagnosis and the histological features of the

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tumor.[4] The main goal of surgery is to completely remove the tumor, leaving no residual malignant cells, while preserving as much function as possible. In cases where there are positive or close surgical margins (tumor within 5 mm of surgical margin), surgical reresection is recommended if the original margins are identifiable and if further surgical resection does not introduce significant functional impairment.[4]

Various studies have demonstrated that the status of the surgical margin may be the single most important factor in the outcome of oral cavity cancer excision, as positive margins are associated with increased complications, recurrence, and morbidity.[5,6] Also, positive margins are more associated with oral carcinoma than with other head and neck tumors.[7,8] The current standard of care is to perform intraoperative frozen section analysis. However, such analysis is typically based on randomly selected tissue biopsies, thus leaving much of the surgical margin unexamined. Furthermore, it has been shown that although the specificity of this technique in predicting the status of margins is very high, the sensitivity is unacceptably low.[9] Additionally, even in cases where the frozen section sample has been reported to be negative, the final histologic evaluation in the immediately adjacent final surgical margin can be positive.[10] Currently there is no standard method of surgical margin analysis when frozen section technique is employed. Other potential disadvantages to the use of frozen section analysis are miscommunication between the surgeon and the pathologist and disorientation of the samples received for frozen section. Finally, frozen section technique can be time consuming and highly expensive, with a cost-benefit ratio of 20:1.[9,10]

The goal of this study was to evaluate the usefulness of optical technology, specifically elastic scattering spectroscopy (ESS), as a diagnostic adjunct to frozen section analysis in patients undergoing surgical resection for squamous cell carcinoma of the oral cavity.

A number of studies have evaluated the usefulness of optical technology for evaluation of normal and abnormal tissue in the oral cavity. McGee et al. demonstrated the significance of anatomy in the production of distinctive spectra from various subsites in the oral cavity. Specifically, a fast excitation emission matrix (FastEEM) spectroscopy device was used to obtain diffuse reflectance, intrinsic fluorescence, and light-scattering spectroscopic measurements (trimodal spectroscopy [TMS]) from different sites in the oral cavity of healthy volunteers, and revealed that intrinsic factors in the anatomy of the oral cavity account for the significant differences observed in the extracted spectral measurements, reflecting the need for the development of subsite-specific spectral algorithms in the oral cavity.[11] A subsequent study by McGee et al. investigated the potential use of FastEEM to distinguish benign from dysplastic/malignant lesions in the oral cavity. Data were collected from patients undergoing biopsy for clinically suspicious lesions and patients undergoing surgical resection of diagnosed malignancy. The results suggested that distinction between benign and dysplastic/malignant lesions was most successful and consistent when anatomy-based algorithms for single subsites, or groups of spectrally similar subsites, were used rather than a single algorithm for all subsites in the oral cavity.[12] Several studies have evaluated the usefulness of optical technologies to detect dysplastic or early malignant lesions in normal-appearing oral mucosa. Tsui et al. used direct fluorescence during the excision of a clinically apparent lateral tongue tumor. Application of this technique resulted in the discovery of visually undetectable but histologically present dysplasia and squamous cell carcinoma up to 25 mm from the clinically detected lesion, even though the surgical margins at 10 mm were histologically normal.[13] A multimodality approach employing optical coherence tomography (OCT) and polarimetry has also been proposed as a screening tool for mapping areas of field cancerization to detect premalignant or malignant lesions in the oral cavity of an animal model.[14]

ESS technology has shown promising results to support its use in settings outside of the head and neck, such as breast cancer,[15–17] Barrett's disease,[18–21] and during colonoscopy.[22,23] It has to date never been investigated as tool to guide surgical resection in the oral cavity.

Optical tissue diagnosis using ESS, mediated by fiberoptic probes, has been shown to be useful to perform noninvasive, or minimally invasive, real-time assessment of tissue pathology in situ.[24]

ESS is a point spectroscopic measurement technique, which is sensitive to cellular and subcellular morphological features.[25–32] Normal and abnormal tissues can generate different spectral signatures as a result of changes in nuclear size, chromatin granularity or density, organelle sizes and densities, and other subcellular features, the optical-spectroscopy equivalent of histopathological readings.[33,34] An important advantage of ESS is that it provides an objective and quantitative assessment of tissue pathology, which does not require on-site special expertise, and may avoid the need for subjective image interpretation as in conventional histopathology.

This study evaluated the potential application of ESS patterns to distinguish benign, dysplastic, and malignant tissue in the surgical margin, which ultimately could improve the prognosis of patients treated for squamous cell carcinoma of the oral cavity.

MATERIALS AND METHODS

Study Subjects and Research Design

This study was conducted at the Boston University Medical Center (BUMC) from 2012 to 2015 and was approved by the BUMC Institutional Review Board. Subjects were recruited from among patients already diagnosed with squamous cell cancer of the oral cavity who were to undergo surgical resection under general anesthesia as part of their treatment. Written informed consent was obtained after thoroughly explaining the study to each subject. In the operating room the surgeon of record determined the resection margin to be used. Spectral readings and biopsies were obtained by the principle investigator or the operating surgeon. Care was taken to clear the mucosa of any surface blood or other debris prior to obtaining spectral readings. Depending on the dimensions of the tumor and the margin, four to 10 spectral readings and biopsies were

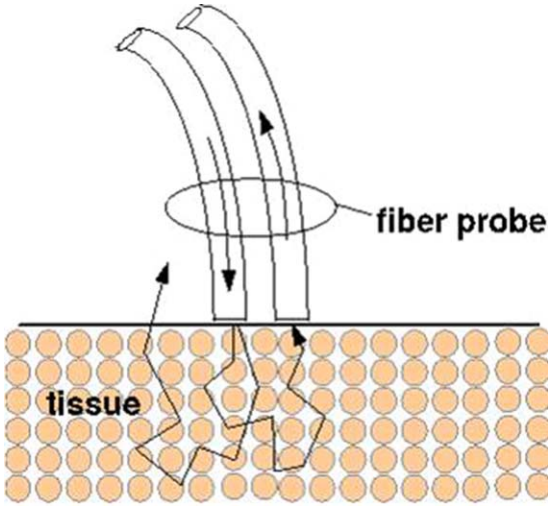

Fig. 1. Schematic diagram of the optical geometry for the elastic scattering spectroscopy fiberoptic tissue measurements. Reprinted with permission from Mourant et al. Elastic scattering spectroscopy as a diagnostic tool for differentiating pathologies in the gastrointestinal tract: preliminary testing *J Biomed Opt* 1996; 1(2):192–199.

taken from each subject from sites that included visible tumor, normal appearing mucosa immediately adjacent to visible tumor, and mucosa at various distances from the edge of the visible tumor up to, but not extending beyond, the planned resection margin. The ESS device was operated by a technician trained in its use. Histological analysis was performed independently by two pathologists blinded to the spectral results. If the two histological analyses differed for any given sample, the two pathologists reviewed that sample together to agree on a single analysis. The spectra obtained intraoperatively were then correlated with the corresponding histological findings.

For each subject the following information was recorded: age, gender, race/ethnicity, TNM stage, tumor subsite (dorsal tongue, lateral tongue, ventral tongue, floor of mouth, buccal mucosa, soft palate, gingiva, hard palate, retromolar trigone), human papillomavirus (HPV) (P16) status, history of tobacco and alcohol consumption, and history and location of prior head neck cancer surgery and/or radiation.

Elastic Scattering Spectroscopy System Description

The method of ESS was developed by Bigio et al.,[27,28,33] and has been demonstrated to have potential clinical use in tissue diagnosis based on the scattering properties of cellular micromorphology.[24,35] ESS is a point spectroscopic measurement technique, over a broad wavelength range (320–900 nm in our current system), which, when performed using appropriate fiberoptic geometry (Fig. 1), has a depth of penetration of 0.5 mm and is sensitive to the microscopic morphological changes occurring in tissues at the cellular and subcellular level. These include nuclear size and density, hyperchromaticity, DNA condensation and chromatin granularity, nuclear crowding, and changes in the size/density of cellular organelles (such as mitochondria) and structural proteins. ESS spectra are derived from the wavelength-dependent optical scattering efficiency and the effects of changes in the scattering phase function (the angular probability distribution) due to the optical index gradients of cellular and subcellular structures) (Fig. 1). As a result of such ultrastructural changes, normal and abnormal tissues generate different scattering spectral signatures, which represent the optical-spectroscopy equivalent of histological appearances. The ESS method senses those morphology changes in a quantitative manner, without actually imaging the microscopic structure.[27,34]

ESS Device Description, Spectra Acquisition, and Biopsy Technique

The ESS device is small, portable, and can be easily carried into the operating room and placed on a standard small utility table near the surgical field (Fig. 2). The fiberoptic probes (core diameter = 200 μm, center-to-center separation = 250 μm) used to obtain the spectral readings were integrated

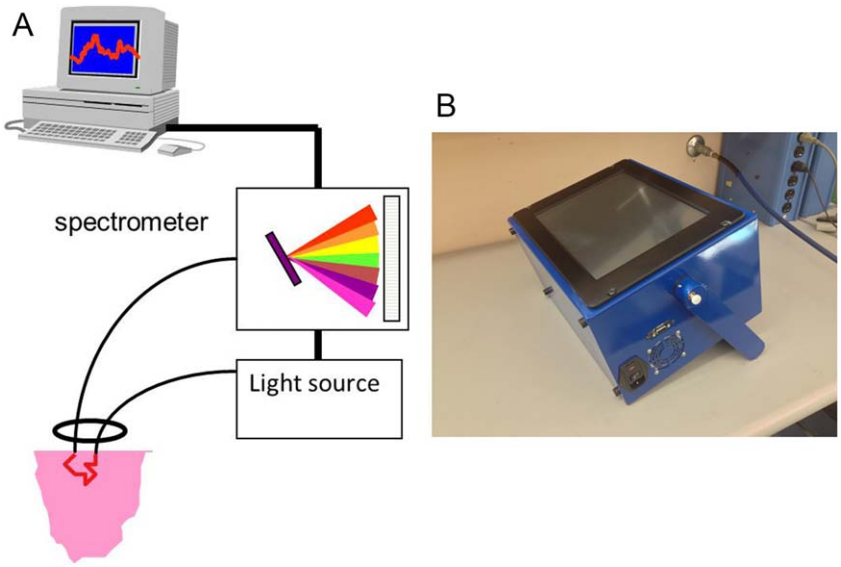

Fig. 2. (A) Schematic diagram of the ESS system. (B) Photo of portable ESS optical biopsy system. ESS = elastic scattering spectroscopy. Reprinted by permission of the publisher (Taylor & Francis Ltd, http://www.tandfonline.com) from Bigio IJ, Brown SG. Spectroscopic Sensing of Cancer and Cancer Therapy: Current Status of Translational Research. *Cancer Biol Ther* 2004;3(3):259–267.

into handpieces custom designed for use in the oral cavity. The diameter of the outer tubing of the fiberoptic probe is 2 mm. Two handpiece designs were made, one straight and one angled. The integrated fiberoptic probe/handpiece units were designed to be sterilized and reused. Prior to recording spectra from the subjects, a calibration spectrum from a spectrally flat diffuse reflection standard (Spectralon; Labsphere, Inc., North Sutton, NH) was taken. The diffuse reflectance of the standard ranges between 250 and 1,000 nm, and can be used to account for spectral variations in the light source, spectrometer, fiber transmission, and fiber coupling. All spectra in this study were recorded as ratios to the Spectralon-reference spectrum, providing data independency from the spectral characteristics of the system. The optical probe was calibrated at the time of the measurement, and the tip of the probe was placed in gentle contact with the tissue site to be investigated. Spectral measurements were obtained by the use of either a foot pedal or the keyboard on the integrated computer. The instrumentation compensates for background light by taking a first measurement without firing the xenon lamp and a subsequent ESS measurement with the pulsed lamp triggered. The ESS spectra used in this study are the result of the subtraction of the background spectrum from the ESS spectrum. Thus, output spectra were calculated according to:

$$I(\lambda)=\frac{I(\lambda)_{tissue}-I(\lambda)_{tissue\ background}}{I(\lambda)_{ref}-I(\lambda)_{ref\ _{background}}}$$

Reproducible and precise measurements were assured by the use of appropriate software that provides graphical illustration of the spectra obtained. Spectrum acquisition for each site measurement and appropriate analysis from the software takes approximately 1 second. After the spectral data are taken, the fiber-probe tip is pressed firmly into the tissue to mark the exact site in the tissue where the spectrum was obtained. A standard 2-mm-diameter biopsy punch (Seamless Premier Uni-Punch; Premier Medical Products Co., Plymouth Meeting, PA) is then used to biopsy the exact tissue site where the spectral reading was taken. Tissue specimens were then fixed in formalin and sent to the pathology laboratory for hematoxylin and eosin staining and histopathological analysis.

Analysis of Spectra

All ESS spectra were preprocessed prior to analysis. Spectral measurements taken at each site before biopsy were averaged, smoothed, and then cropped, resulting in spectra encompassing 216 wavelength bands, from 330 to 760 nm. These spectra were then normalized to the intensity at 650 nm to enhance spectral shape, not relative intensities. To classify measured spectra, a diagnostic algorithm based on multidimensional pattern-recognition/machine learning was developed. Given the high-dimensional nature of the data, a framework consisting of dimensionality reduction followed by classification was used. Sequential floating forward selection was used for dimensionality reduction,[36] followed by multidimensional classification using linear support vector machines.[30,37–39] The diagnostic algorithm was designed to have as an output a score that relates to whether or not the input spectrum is found to be malignant or benign by the algorithm. This output is referred to as the malignancy score, which has a value in the range of 0 to 1. The closer the score is to 1, the higher the chance that the spectrum was obtained from a malignant sample, and, conversely, the closer the score is to 0 the higher the chance it is from a benign sample. Leave-one-out cross-validation was used to optimize classifier parameters and obtain classification performance estimates. Sensitivity and specificity, error rate, and area under the curve were used as performance metrics for tests involving classification between disease and nondisease classes.[40] Statistical analysis was performed using the Fisher exact test and the Bonferroni corrected multiple comparison test where appropriate.

RESULTS

Subject Data

Thirty-four subjects were enrolled in the study, 15 males and 19 females, with a mean age of 59 years (range, 25–81 years). Twenty-seven subjects (79%) were Caucasian, one (3%) was African American, four (12%) were Hispanic, and two (6%) had no race/ethnicity recorded. Almost half (47%) had a history of ethanol use, and 71% had a history of tobacco use. Of the 15 subjects who were tested for HPV status, five were positive. The majority of subjects (75%) had advanced stage (III, IV) disease. The most common subsite involved in the oral cavity was the lateral tongue (15 subjects), followed by the floor of mouth (12 subjects), and gingiva (seven subjects). Only one patient had prior surgery, and no patients had radiation to the oral cavity. Subject data are summarized in Table I.

Correlation of Spectral Data With Pathology

Twenty-seven subjects had spectroscopic data that could be analyzed. Seven subjects were excluded from this part of the study, either because biopsies were not obtained (e.g., case aborted) or spectral data were not available (e.g., technical difficulty with obtaining or processing the data).

One hundred seventy-six (176) spectral data point/biopsy specimen pairs were available for analysis. Table II shows the distribution of pathological findings in these 176 biopsies.

Several comparisons were made to determine the effectiveness of ESS to discriminate between the various pathologic categories outlined in Table II. ESS distinguished normal tissue and mild dysplasia from severe dysplasia, carcinoma in situ (CIS), and invasive cancer, with sensitivity 84% and specificity of 71%. The receiver operating characteristic curve for this comparison is shown in Figure 3. ESS distinguished normal tissue and mild dysplasia from moderate dysplasia, severe dysplasia, and CIS with a sensitivity of 100% and specificity 89%. ESS also distinguished normal tissue from mild, moderate, and severe dysplasia and CIS with a sensitivity 85% and specificity 78%. These data are summarized in Table III.

For normal tissue and mild dysplasia versus moderate dysplasia, severe dysplasia, and CIS, and for normal tissue versus mild, moderate, and severe dysplasia and CIS, the differences in the accuracies of the diagnostic algorithms were not found to be statistically significant ($P = .06$, Fisher exact test).

Impact of Inflammation

Several comparisons were also made to determine if the presence of inflammation affected the ability of ESS to discriminate between the various pathological categories. ESS distinguished histologically normal tissue

TABLE I.
Subject Data

Subject Data (N = 34)	Value
Gender	
Male	15
Female	19
Race/ethnicity	
Black/African American	1
White/Caucasian	27
Hispanic/Latino	4
Unknown/not reported	2
Mean age, yr	59 (range, 25–81)
HPV status (P16)	
Positive	5
Negative	10
Unknown	19
Cancer stage	
1	5
2	3
3	2
4	22
Stage unknown	2
Ethanol use history	
Yes	16 (12 current)
No	17
Unknown	1
Mean drinks per day (n = 16)	4 (range, 0.5–12)
Tobacco use history	
Yes	24 (9 current)
No	8
Unknown	2
Mean pack-years (n = 24)	26.7 (range, 2–80)
Oral cavity subsite involved by tumor (some tumors involved more than 1 subsite)	
Buccal mucosa	3
Gingiva	7
Dorsal tongue	2
Floor of mouth	12
Hard palate	1
Lateral tongue	15
Retromolar trigone	3
Soft palate	0
Ventral tongue	4
Prior radiotherapy to oral cavity	0

HPV = human papillomavirus.

without inflammation from invasive cancer with inflammation (chronic or mixed chronic/acute) with a sensitivity of 86% and specificity of 73%, and distinguished histologically normal tissue with inflammation from invasive cancer with inflammation with a sensitivity of 83% and specificity of 74%. These data are also summarized in Table III. The differences in the accuracies of these diagnostic algorithms were not found to be statistically significant ($P = .50$, Fisher exact test).

TABLE II.
Pathologic Diagnoses

Pathologic Diagnoses	No. of Biopsy Specimens
Normal tissue	91
Mild dysplasia	7
Moderate dysplasia	2
Severe dysplasia/carcinoma in situ	4
Invasive cancer	72
Total	176

Malignancy Score

Malignancy scores were developed from the classification generated by the analysis in Table III. The higher the value of the score, the higher the likelihood of malignancy. Figures 4 and 5 show the malignancy scores for the various pathologic and inflammation grades, respectively. For the pathology grades, there was a statistically significant difference between normal tissue and invasive cancer. For the inflammation grades, there was a statistically significant difference between noninflamed tissue and chronically inflamed tissue, and between noninflamed tissue and tissue with mixed (chronic and acute) inflammation.

DISCUSSION

Patients diagnosed with squamous cell carcinoma of the oral cavity typically undergo surgical excision with 10-mm margins. The goal is to achieve surgical margins with no residual malignant cells because positive surgical margins are associated with increased risk of morbidity.[5,6] Sutton et al. reported that patients with close surgical margins (tumor < 5 mm from surgical margin) after resection of oral squamous cell carcinoma had significantly higher rates of perineural invasion and vascular invasion compared to patients with negative surgical margins. Additionally, in those patients with close surgical margins, the tumor's characteristics demonstrated greater diameter and a more aggressive invasion pattern with nodal metastatic disease.[41] Another study also demonstrated that a higher incidence of recurrence at the primary site and significantly lower 5-year survival rate were observed when tumor was present or close (<2 mm) to the surgical margins as compared to clear surgical margins.[6] Moreover, patients with oral cavity cancer are at increased risk for local recurrences and the development of second primary tumors, even after surgical excision of the primary malignancy. This observation could be explained by a phenomenon known as field cancerization, which was first described by Slaughter et al. almost 6 decades ago.[42] In this study of 783 patients with oral cavity carcinoma, 88 instances of independent multiple tumors were detected, which was far beyond the statistical possibility of chance occurrence. The group suggested that field cancerization is a significant factor in local recurrences and multifocality ("satellite" or "skip" lesions) of oral cavity tumors. Recent studies also strongly support the dynamics of the field cancerization theory in the oral cavity.[43,44] According to these studies,

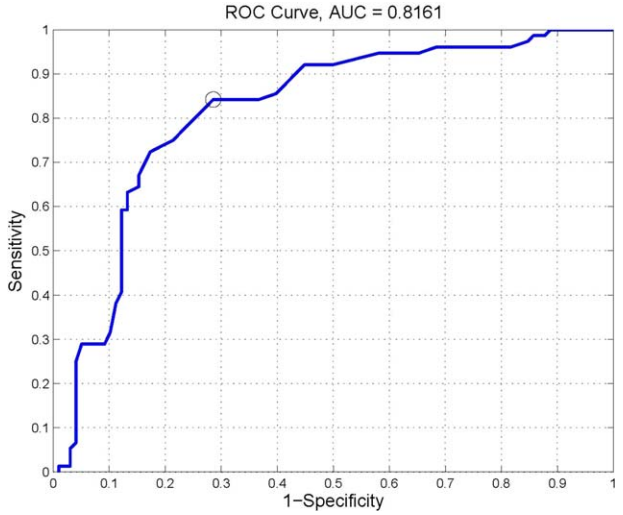

Fig. 3. Sample ROC curve for comparison group 1. AUC = area under the curve; ROC = receiver operating characteristic. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

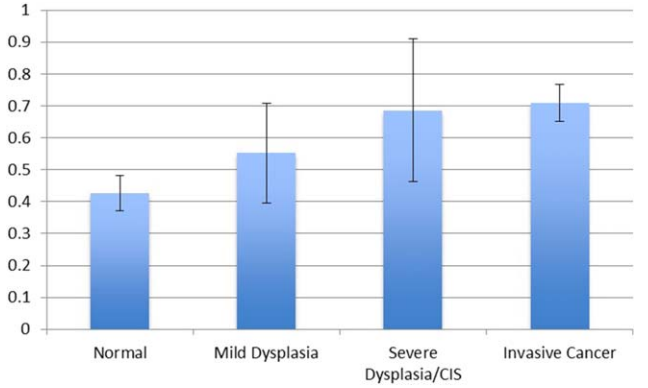

Fig. 4. Malignancy score—pathology. CIS = carcinoma in situ. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

local recurrence of excised aggressive oral cavity carcinoma can be observed even in cases where clear surgical margins, as determined by histopathology, were achieved.[43] Additionally, Tabor et al. reported that squamous cell carcinoma in the oral cavity may show common clonal origin with second primary oral cancer, even if the distance between them was >7 cm, and the mucosa separating them appeared visually and histologically normal. These results indicate that large fields of healthy mucosa are replaced by a population of genetically altered cells of monoclonal origin.[44] Modern molecular techniques, such as measuring the loss of heterozygosity with microsatellite analysis, demonstrate genetic alterations associated with malignant conversion, despite the fact that biopsies from surgical margins of excised oral cavity carcinoma were determined to be histopathologically normal.[45] Nevertheless, frozen section analysis remains the current standard of care for the intraoperative detection of safe surgical margins during excision of squamous cell carcinoma in the oral cavity. However, this technique can be unreliable in distinguishing benign from malignant tissue because it can have a high false negative rate.[9,10] Furthermore, it typically samples only a small area of the entire mucosal margin.

Achieving negative margins in oral cavity cancer resection may be the only factor related to recurrence that is within the control of the surgeon. Discovery of newer, faster, and more reliable methods to monitor surgical margins are therefore critical in aiding surgeons. The ideal system for intraoperative assessment of surgical margins is one that can be integrated clinically without disrupting the flow of the surgical procedure, that can rapidly provide real-time, accurate assessment of margins, and that does not require on-site special expertise other than the surgeon. To this end, various optical technologies have been studied. Early work by Backman et al. showed that light scattering spectroscopy (LSS) has the potential to detect epithelial precancerous lesions and preinvasive cancers in various organs throughout the body, including the oral cavity.[46] Subsequently, work by Muller et al. and McGee et al. using a FastEEM device with TMS, which utilizes diffuse reflectance and intrinsic fluorescence measurements in addition to LSS, showed promising results in distinguishing histologically normal tissue from dysplastic/malignant tissue and benign lesions from dysplastic/malignant lesions in the oral cavity but is dependent on anatomic subsite-based algorithms.[11,12,47] Furthermore, the device is large and not easily transportable to the operating room, and spectral readings can take more than a few seconds to record and can thus be distorted if the contact probe moves during the recording process. Direct fluorescence has also shown promise in detecting occult

TABLE III.
Results of ESS in Distinguishing Between Various Comparison Groups.

Pathology/ESS Comparison Groups		Sensitivity	Specificity	Total Error	PPV	NPV	AUC
1	N/MiD vs. SD/CIS/IC	0.8421	0.7143	0.2299	0.6957	0.8537	0.8161
2	N/MiD vs MoD/SD/CIS	1.0000	0.8878	0.1058	0.3529	1.0000	0.9354
3	N vs. MiD/MoD/SD/CIS	0.8462	0.7802	0.2115	0.3548	0.9726	0.8453
4	N(NI) vs. IC(I)	0.8592	0.7273	0.1720	0.9104	0.6154	0.8025
5	N(I) vs. IC(I)	0.8310	0.7391	0.2143	0.7662	0.8095	0.8048

AUC = area under the curve; CIS = carcinoma in situ; ESS = elastic scattering spectroscopy; IC = invasive cancer; IC(I) = invasive cancer with inflammation (chronic or mixed chronic/acute); MiD = mild dysplasia; MoD = moderate dysplasia; N = normal tissue; N(I) = normal tissue with inflammation (chronic or mixed chronic/acute); N(NI) = normal tissue with no inflammation; NPV = negative predictive value; PPV = positive predictive value; SD = severe dysplasia.

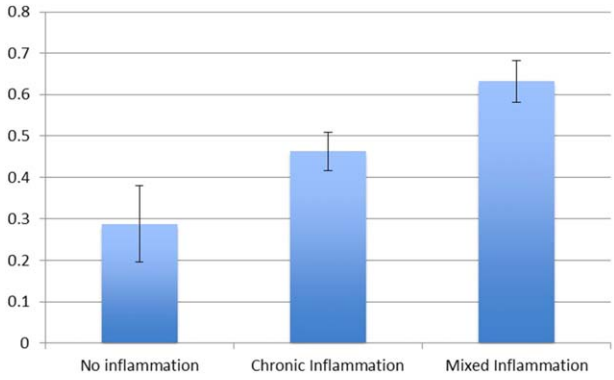

Fig. 5. Malignancy score—inflammation. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

premalignant and malignant disease in the oral cavity in a handful of patients, but more studies are needed to determine its effectiveness.[13,48] OCT has also been shown to be useful in detecting premalignant malignant lesions in the oral cavity of animals and humans.[14,49] However, the depth of penetration can be limited by other factors such as absorption, and it requires special training and expertise to interpret the images. Furthermore, the speed of data acquisition is slow; thus, OCT is sensitive to any movement of the tissue or probe.

There are several important differences between ESS and other optical methods for assessing oral cancer. The information contained in the ESS spectrum most closely correlates to the microstructural and architectural changes that a histopathologist looks for but without the need for image interpretation. In contrast, fluorescence spectroscopy, and in particular autofluorescence, assesses biochemical changes and can be affected by variations in the local pH and electronegativity as well as by blood oxygenation.[50] Autofluorescence spectra can also be influenced by factors such as gender, pigmentation, tobacco use, alcohol consumption, and the wearing of dentures.[51] Moreover, although ESS can be performed in the presence of full room light, autofluorescence spectroscopy and Raman spectroscopy generally require temporary reduction or complete elimination of ambient light, which may be impractical in clinical settings.

Diffuse reflectance spectroscopy (DRS) has also been investigated as an optical method to assess oral cancer. Einstein et al. used DRS to study large, excised tissue samples.[52] However, the analysis was based on assumptions of the diffusion approximation to transport theory. Diffuse reflectance requires measurement of optical reflectance from large tissue volumes, whereas the sensing volume of ESS is much smaller, such that ESS can detect changes in tissue micromorphology on a millimeter range spatial scale, and thus it is better suited to assessing the precise location of changes at margins.

Confocal reflectance microscopy (CRM) has also been studied as a nonspectroscopic optical approach to in situ assessment of oral mucosa. The large size of early CRM devices limited study to the lip and tongue. More recently, Maitland et al. used a miniature microscope to apply CRM technology to oral tissue.[53] This was demonstrated in vivo to provide moderate-resolution microscopic images of oral mucosa, aided by an applied acetowhitening agent to increase scattering from nuclei. This method images features that can be correlated with conventional histopathology findings and shows promise for assessing resection margins. However, similar to OCT, real-time interpretation of the microscopic images would require the surgeon to develop the skills to read the images and render subjective interpretation. In contrast, the ESS method provides an objective, quantitative determination of the tissue microarchitecture, rendered instantaneously and not requiring subjective interpretation.

ESS has shown promising data in areas of the body other than the oral cavity.[15–23] To our knowledge, this study is the first to evaluate ESS technology to guide surgical resection in the oral cavity. The main goal was to evaluate its effectiveness in distinguishing normal, dysplastic and malignant tissue in the oral cavity.

The ESS system used was lightweight, portable, and could be easily transported to the operating room. The custom made handpieces facilitated easy access of the fiberoptic probe to all areas of the oral cavity. Spectral readings were obtained typically in under 1 second. With a depth of penetration of 0.5 mm, the system is well suited to evaluate oral cavity mucosa, which varies in thickness from about 100 to 300 μm. The ESS system is not commercially available at the present time. However, if produced in large numbers, the ESS system would cost approximately $10,000 and the disposable fiberoptic probes approximately $20 each.

ESS effectiveness was calculated by grouping several different pathologic categories rather than comparing single pathologic categories. This was done for two reasons. First, such groupings often reflect real clinical situations. For example, during resection a surgeon may be most interested in distinguishing severe dysplasia from mild dysplasia but not severe dysplasia from CIS, because in the latter case, both would need to be resected to safely achieve a negative margin, whereas leaving mild dysplasia at the margin may be acceptable. Second, there was a paucity of specimens with dysplasia and CIS, making it difficult to provide meaningful comparisons without combining some groups.

ESS was effective in distinguishing normal and mildly dysplastic tissue from severe dysplasia, CIS, and invasive carcinoma and from moderate dysplasia, severe dysplasia, and CIS with a sensitivity/specificity of 84%/71% and 100%/89%, respectively. These are clinically relevant comparisons, because most surgeons will resect additional tissue if there is severe dysplasia or CIS at the margin, but there are instances where it is not feasible to "chase" mild dysplasia.

ESS was also effective in distinguishing histologically normal tissue from all grades of dysplasia and CIS. This is a clinically relevant comparison because dysplasia and CIS often occur in visibly normal mucosa, and the major challenge for surgeons in determining resection margins is in distinguishing visibly and histologically

normal tissue from visibly normal but histologically abnormal tissue. Although these results are encouraging, it is important to note that the number spectral data point/biopsy specimen pairs was small, particularly for the various grades of dysplasia and CIS. For normal tissue and mild dysplasia versus moderate and severe dysplasia and CIS, and for normal tissue versus mild, moderate, and severe dysplasia and CIS, the differences in the accuracies of the diagnostic algorithms, although not statistically significant, had a *P* value close to.05, suggesting that more significant differences might be noted with more data points. Furthermore, we were unable to determine from this study whether the accuracy of ESS would have been improved further by analyzing the above comparisons for each anatomic subsite separately, because the number of data points/biopsy specimen pairs available for each subsite was not sufficient.

Inflammation is often a confounding factor in optical diagnosis, particularly with those technologies that include assessment parameters such as vessel size or hemoglobin saturation, because these can change significantly in inflammation. Inflammation did not appear to have a significant effect on the ability of ESS to distinguish between normal tissue and invasive cancer, regardless of whether the normal tissue was inflamed or not. This may be due to the fact that ESS technology relies primarily on parameters such as nuclear size and density, hyperchromaticity, and chromatin granularity, which may be less affected by inflammation.

The malignancy scores for the various pathology grades further reflect the effectiveness of ESS in distinguishing benign, dysplastic, and malignant tissue in the oral cavity. Although the only statistically significant difference seen was between normal mucosa and invasive cancer, it is possible that significant differences between normal mucosa and moderate/severe dysplasia/CIS would be seen with a larger sample size for the latter group. The difference in malignancy scores between noninflamed tissue and tissue with chronic or mixed inflammation is an interesting finding, but the significance of this is unclear. It may reflect the fact that more malignant specimens showed inflammation compared to normal tissue specimens.

There are several limitations to this study and to the ESS system itself. First, the presence of blood on the surface mucosa may have affected spectral readings. Even with careful irrigation and sponging, it was sometimes difficult to keep the surface completely free of blood. In several cases the margins were incised by the surgeon prior to the taking biopsies, and this added to the problem of surface blood. Second, deep margins were not assessed. It has been shown that most positive margins occur at the deep margin in oral cavity cancer.[8] It is not known how effective ESS would be in evaluating deep margins, and this will require further study. Third, there were only a few biopsy specimens showing dysplasia, and it was not always known if these came from visibly normal or abnormal tissue. Clinically, the most important application of optical technologies like ESS is their potential to detect severe dysplasia, CIS, or even microinvasive cancer in visibly normal mucosa. To determine this, a much larger sample size of dysplastic and CIS biopsies from normal-appearing mucosa would be necessary. Finally, the current ESS technology utilizes a fiber-based contact probe, which can only assess a 2-mm-diameter area at a time. Even though spectral readings can be obtained in less than 1 second, the small probe size and need for tissue contact make it impractical to assess the entire mucosal margin in most cases. A device (for example, a noncontact imaging device) that utilizes EES technology and is capable of rapidly assessing many millimeter-range surface areas simultaneously would allow assessment of a larger surface area and would be more practical in assessing margins.

CONCLUSION

This is the first study to evaluate the effectiveness of ESS in guiding mucosal resection margins in oral cavity cancer. ESS provides fast, real-time assessment of tissue without the need for pathology expertise. ESS appears to be effective in distinguishing between normal mucosa and invasive cancer and between normal tissue (histologically normal and mild dysplasia) and abnormal tissue (severe dysplasia and CIS) that might require further margin resection. Further studies, however, are needed with a larger sample size to validate these findings and to determine the effectiveness of ESS in distinguishing visibly and histologically normal tissue from visibly normal but histologically abnormal tissue.

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