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Assessment of oral premalignancy using elastic scattering spectroscopy

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Soft tissue

Summary Optical spectroscopy systems have been involved in various clinical fields; however the main interest is still in the diagnosis of premalignant/malignant lesions. The aim of this study was to compare findings of Elastic Scattering Spectroscopy (ESS) with histopathology of oral tissues to see if this technique could be used as an adjunct or alternative to histopathology in identifying dysplasia. The technique involves the use of Mie scattering and is a simple non-invasive method of tissue interrogation. Twenty-five oral sites from 25 patients who presented with oral leukoplakia were examined by ESS using a pulsed xenon-arc lamp. Surgical biopsies were acquired from each of the examination sites. The results of the acquired spectra were then compared with histopathology. Two sets of spectra were obtained, and by using a linear discriminant analysis, a sensitivity of 72% and a specificity of 75% were obtained. These results are promising and could suggest that ESS may be able to identify dysplasia in oral tissues. To prove the usefulness of the ESS in dysplasia detection in oral tissues conclusively, a larger body of data is needed. We aim to continue this study to obtain more data in an attempt to increase the accuracy of the technique. Large, multi-centre trails are needed for each anatomical site, in order to gather more information about the differences between normal and dysplastic tissue.

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Introduction

Elastic Scattering Spectroscopy (ESS) is an emerging technique that generates a wavelength dependant spectrum that reflects structural and morphological change within tissues. Elastic scattering implies that the light returns with the same kinetic energy as the incident photons. The incident light can undergo single, or more commonly, multiple scattering events before being collected again at the same surface by an optical probe and the resulting data is analysed. The acquired data reflects both the scattering and absorptive properties of that tissue.[1] This scattering process has been shown to occur at gradients in the optical index of refraction resulting from differences in densities that occur at a cellular and sub-cellular level. The structures that induce the scattering (collectively known as scattering centres) are the nucleus, chromatin concentration, and sub-cellular organelles; other scattering centres include structural proteins, lipids and erythrocytes. ESS has been shown to be sensitive to nuclear size, chromatin content and nuclear/cytoplasmic ratio which are all criteria that the histopathologist looks for when establishing malignancy within a tissue.[2–6]

ESS has the advantage of being fast, reliable and cost effective and potentially offers a non-invasive diagnosis in situ, and in real-time. The technique has not been used only in the diagnosis of dysplasia and malignancy,[5,7–10] but can be used to monitor chemotherapy levels and free-flap oxygenation levels. It can also enable guided rather than random biopsies, and also assess surgical margins and regional lymph nodes intra-operatively. ESS has, in a number of studies, been combined with extracted intrinsic fluorescence and diffused reflectance forming a ''tri-modal spectroscopy'' device which has been shown to have a higher sensitivity and specificity when examining premalignant and malignant lesions.[11]

Therefore, the aim of this clinical trial was to evaluate the ability of the ESS system to discriminate potentially malignant lesions from normal tissues in the oral cavity and to develop an algorithm for the precise analysis of retrieved tissue signals.

Materials and methods

Twenty-five patients, 13 males and 12 females, (mean age 52 years, range 41–67 years) with clinically suspicious oral leukoplakia took part in this study in the Maxillofacial Unit, University College Hospital (UCH), London. The trial protocol was approved by the joint UCL/UCLH committees of the ethics for human research.

An information sheet explaining the aim of the study in simple non-scientific terms was given to each patient who was then consented prior to examination. ESS was used to examine the suspicious area of each of those patients prior to surgical biopsy. Inclusion criteria were patients over 18 years of age who presented with one or more suspicious oral lesion.

Table 1 The breakdown of oral tissues in the dataset

Site in oral cavity	No. of biopsies
Buccal mucosa	9
Floor of mouth	3
Sublingual	2
Tongue	11
Total	25

Table 2 The breakdown of histological grades in the dataset

Histology	Freq. of occurrence
Normal	4
Hyperkeratosis	10
Mild dysplasia	6
Moderate dysplasia	3
Severe dysplasia	1
Carcinoma in situ	1
Total	25

The 25 biopsies were taken from various oral sites (Table 1), and examined histopathologically (Table 2). In this clinical trial, all types of dysplasia were classified as 'malignant' whereas all other reports (normal, inflammation, and hyperkeratosis) were considered ''non-malignant or benign'' changes.

The ESS system

The ESS system (Fig. 1) have been described in previous publications,[1,2,9–11] is a prototype that was designed and built at the Los Alamos National Laboratory, USA. The system consists of a pulsed xenon-arc lamp for the light source, a PC-compatible spectrometer, which employs a linear charged coupled device (CCD) array for detection, an optical fibre (graded-index) based probe, and a laptop computer for system control and data display. The wavelength range of the system is 300–900 nm, but the range used for these studies was 330–750 nm, which covers the near UV and visible part of the electromagnetic spectrum; the range of the spectrum usually covers the light emitted by cellular and sub-cellular organelles. The output of the arc lamp is coupled to the illumination fibre, which has a core diameter of 400 μm. A second, 200 μm diameter fibre alongside the illuminating fibre, collects the scattered light from the tissue and guides it to the spectrometer where an optical spectrum is generated for further processing; centre-to-centre separation between the illuminating fibre and collecting fibre is 300 μm. In this configuration, any photon from the illumination fibre that reaches the collection fibre must have undergone one or more scattering events in the tissue. A reflectance standard was used in the procedure to account for the spectrum of the xenon-arc lamp.

The system probe (Fig. 2) was designed to be used in (gentle) optical contact with tissues, and

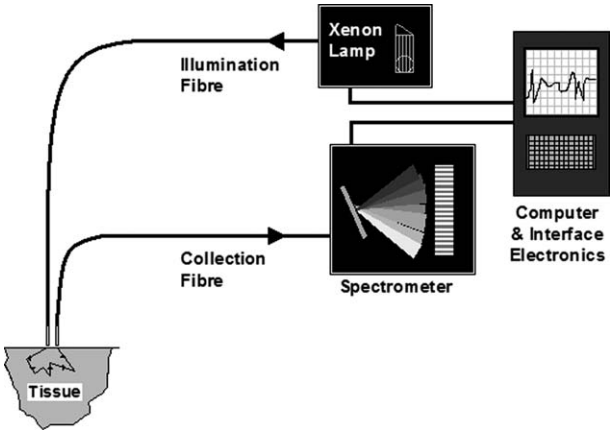

Figure 1 Schematic diagram of ESS diagnostic system.

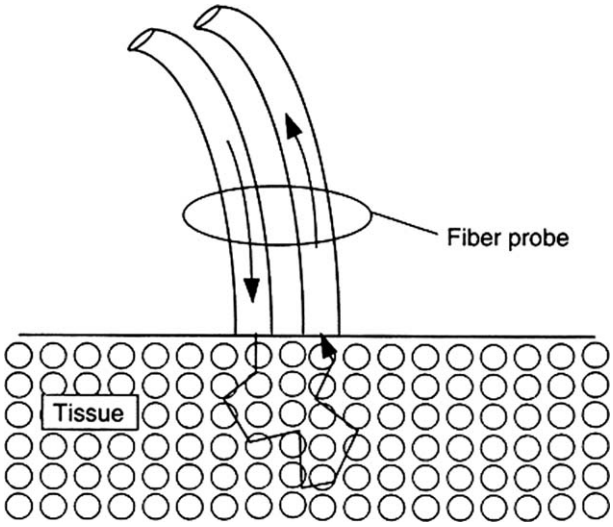

Figure 2 Schematic diagram of the fibre optic probe.

incorporates two optical fibres, one to transmit the light into the tissue and the other one for collecting the scattered light from tissue; the two probes are built-in one bigger probe so the viewer can see only the latter. Placement of the probe in direct contact with the tissue avoids interference with specularly reflected light. With this probe geometry, the volume of tissue visited by the collected photons occupies $\approx$1 mm^3. This has been determined from computational simulations using Monte Carlo code, which incorporates Mie theory for the details of the scattering events.

In clinical use, the tip of the fibre probe was momentarily placed in direct contact with the suspected lesion, and the measurement was activated at the keyboard. The system automatically takes a background measurement without firing the lamp, followed immediately (within-100 ms) by an ESS measurement with the pulsed lamp was been triggered, and then subtracts the background spectrum from the ESS spectrum. The entire measurement processes (activating the spectrometer, triggering the arc lamp, reading the detector array with an analogue/digital (A/D) converter etc.) were computer controlled by a laptop PC. This allowed both accurate and reproducible measurements within the clinical setting. It provides also the clinician with the advantage of rapid data acquisition and a display time of less than 1 s for each site measured. Three optical measurements were acquired from each of the suspected lesions; 1st measurement from the centre, 2nd from the periphery of the lesion and in between the two measurements the 3rd was acquired.

Following the optical readings, a surgical biopsy was taken, preserved in formalin, processed in H&E stain and examined by the histopathologist; digital

pictures have been taken and sketches have been made for all the suspected sites to ensure that all sites are easily identified by the pathologist and hence reduce any chance of false results. The tissue samples (25 biopsies in total) were graded as normal, hyperkeratotic, mild dysplastic, moderate dysplastic, severe dysplastic and carcinoma in situ. The elastic spectrum of each lesion was correlated to its histology.

In this study, spectra were combined together although they originated from histologically different oral sites (floor of mouth, tongue, cheek); before doing this, each acquired spectrum from a particular site was compared to a spectrum acquired from a histology-similar normal oral site; the intention here was to reduce discrepancies in the readings.

Data analysis

The number of dimensions in the data (wavelengths) greatly exceeded the number of observations (spectra), and so a naive analysis of the raw data would result in a model that was vastly overfitted and would be much more sensitive to noise in the training data set. To avoid this problem, the data was cropped between 340 and 800 nm to remove those parts of the spectrum with low signal-to-noise ratio. Spectra were smoothed using a Savitsky–Golay linear filter (to remove the noise) with a smoothing width of 30 points (10 nm), and normalised so that each spectra had a mean intensity of zero and a standard deviation of unity with virtually no loss of information; by doing this we were able to compare and analyse spectra with different intensities.

A linear discriminant analysis was used to find a transformation that maximised the difference between the data belonging to the dysplastic and the benign groups. In order to determine how successful this algorithm was at discriminating between the novel benign and dysplastic spectra, leave-one-out (or ''jacknife'') validation was used. All analyses were performed in R (R Development Core Team, 2005), using software libraries from (Venables and Ripley 2002).

Results

The results of this study represent the first assessment of the use of elastic scattering spectroscopy in the classification of oral tissues. A total of 75 spectra were taken. The mean spectra of dysplastic and normal sites are shown in Figure 3. Moreover,

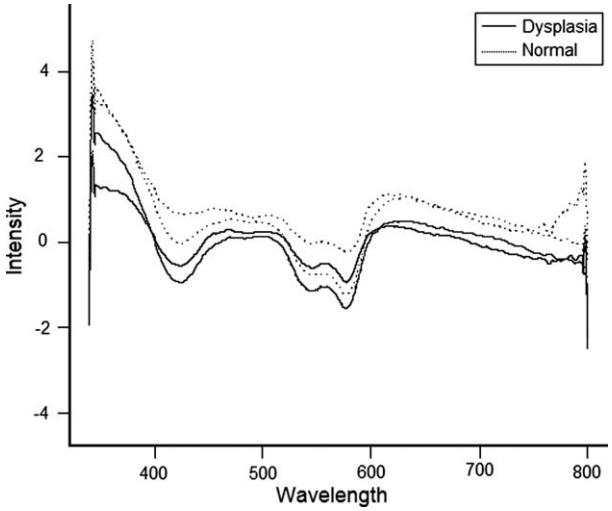

Figure 3 A representative plot of the mean dysplastic and benign spectra (solid lines), with one standard deviation plotted either side of the mean (dashed lines).

the standard deviations of the spectra were large and tend to overlap, although differences in shape of the mean scattered spectra were apparent. The application of an ROC Curve (Receiver–Operator Curves) in Figure 4 suggests that the trade-off between optimal sensitivity and specificity is 0.727 and 0.75, respectively. Table 3 shows the value true positive, true negative, false positive, and false negative, a sensitivity of 72.7% and specificity of 75% were obtained.

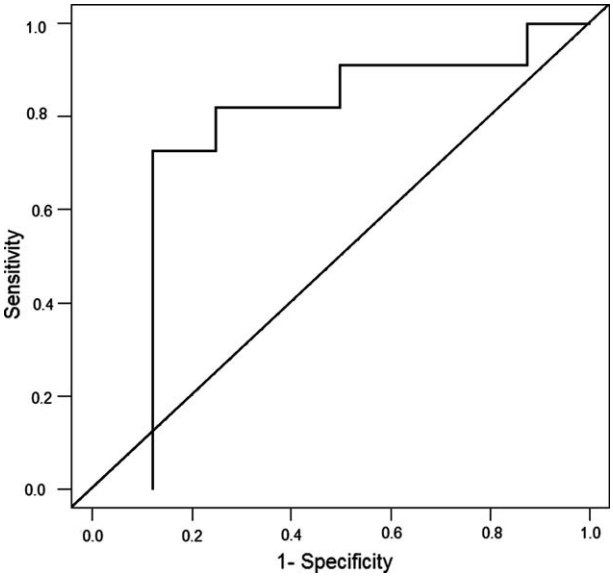

Figure 4 A ROC curve (Receiver–Operator Curve) computed for distinguishing between benign and dysplastic oral lesions by the trade-off between sensitivity and specificity. Optimal sensitivity (0.727), optimal specificity (0.75).

Table 3 Classification of oral tissue ESS spectra (dysplasia vs. normal tissue)

Number of biopsies showing dysplasia histologically	11
Number of dysplastic sites diagnosed by ESS as dysplasia (true positive)	8
Number of dysplastic sites diagnosed by ESS as normal (false negative)	3
Sensitivity (%) = TP/(TP + FN)[a]	72.7%
Number of biopsies found histologically to be normal	4
Number of normal ESS spectra found histologically to be normal (true negative)	3
Number of normal ESS spectra found histologically to be dysplastic (false negative)	1
Specificity (%) = TN/(TN + FP)[a]	75%

[a] Where TP, TN, FP, FN represent true positives, true negatives, false positives and false negatives, respectively.

Discussion

The use of a new diagnostic instrument for oral dysplasia is a real challenge, especially since no database is available. The oral tissue, which includes keratinized, non-keratinized, glandular and fatty tissue, results in variable signals. These render the plotting and correlation of the obtained data set more complicated.

Recently, there has been increasing interest in the use of optical spectroscopy systems to be able to provide tissue diagnosis in real-time, non-invasively and in situ. These systems rely on the fact that the optical spectrum derived from any tissue will contain information about the histological and biochemical make up of that tissue. The technique has not only been shown to have a role in the detection of dysplasia and malignancy but also in performing guided biopsies, monitoring of haemoglobin tissue perfusion in free-flaps and therapeutic drug levels during chemo- and photodynamic therapy.[12,13]

Epithelial dysplasia in the gastrointestinal tract and, in particular Barrett's esophagus is important disease states that is being currently investigated by ESS. A number of promising clinical studies have shown some unequivocal results suggesting that this technique can be used as an adjunct to histopathology since it is a cheap, simple-to-use tool and can give rapid accurate diagnoses.[11,14–18]

Badizadegan et al.[11] reported that there are ongoing clinical studies investigating pre-cancer in the oral cavity, the uterine cervix and the gastrointestinal tract. Georgakoudi and Feld[19] used trimodal spectroscopy in patients with Barrett's esophagus and obtained higher sensitivity and specificity when compared with one of the combined techniques.[15] Work by Haringsma,[20] on Barrett's oesophagus found that ESS was superior than other optical diagnostics in terms of early detection of low-grade dysplasia, based on structural information of the mucosa, in which the size and crowding of nuclei in the epithelial layer play a key role.

The study of malignancy and dysplasia of the oral cavity using ESS is in development. Ex vivo work by Jerjes et al.[9] used the technique to study formalin-fixed specimens of cervical lymph nodes and bony margins taken from patients with oral squamous cell carcinoma. Using linear discriminant analysis they showed sensitivity and specificity of 98% and 68%, respectively for the lymph nodes and 87% and 80% for the bone margins. The same group suggested that ESS has the potential to perform a full optical mapping of the suspicious area, thus eliminating the need for pathology.[10]

Muller et al.[21] used ESS to look at normal versus abnormal tissue and dysplastic versus cancerous in the oral cavity. When comparing spectroscopy to histopathology, the accuracy for normal tissues was 91.6% (22/24) compared to 97% (33/34) for abnormal tissues. These figures fell when examining dysplasia, 64.3% (9/14) and carcinoma 50% (5/10). However, when using tri-modal spectroscopy, Muller et al showed a sensitivity and specificity of 96% when comparing cancerous/dysplastic tissues from normal tissues and obtained values of 64% and 90%, when comparing dysplastic with cancerous tissues, respectively.

Compared with previous studies using light to differentiate between normal and benign lesions on other human tissues, and despite the small number of cases plus the heterogeneity of the oral tissues, our results yield a reasonable outcome. A small trial examining the combination of diffuse reflectance spectroscopy with fluorescence spectroscopy for the detection of cervical pre-cancer suggests that the two techniques provide complementary diagnostic information.[8]

Overall, the results of this study considered being the first step on the right tract for developing a non-invasive, spectroscopic method for evaluating oral lesions. Although this early result is promising, it is unlikely to hold if more data is added to the analysis, since in this small dataset, patient, site, and histological grade are all confused, and it is likely that the high level of discrimination

between benign and dysplastic tissues is due to the correlation between histological grade and, for example, patient. Consequently, it may that the results shown here in fact discriminate between patients, rather than histological grade. However, further work is required in the form of the collection of more data sets, and careful analysis using multivariate statistical techniques on all the available spectral information, which can potentially improve the accuracy of detection. This requires multiple samples within each tissue category and a large number of patients before it can be used in routine oral diagnosis.

Some of the 28% and 25% reduction in the sensitivity and specificity, from 100% can be accounted for by the possibility of discrepancy occurring from combining spectra from different anatomical sites and also could be related to the co-registration of the suspected site.

Conclusion

The ''gold standard'' of assessing pathological changes in tissue is currently histopathology. However the processing of biopsy material and the interpretation of the results inevitably leads to diagnostic delay and the added problem of taking an unrepresentative sample.

The field of head and neck oncology is increasingly using light spectroscopy techniques to not only diagnose dysplasia and malignancy but also to monitor treatment and potential complications. These techniques have been shown to be able to provide a diagnosis non-invasively, in situ and in real-time.

At present, this preliminary study is promising; large multi-centre trials will offer more patients and hence more data that could possibly improve the sensitivity and specificity of this technique. This also could be improve by more accurate co-registration of the suspicious sites and avoid any technical difficulties.

The ultimate aim is to be able to accurately diagnose pathology without the need to remove a tissue sample, diminish patient trauma as well as financial implications.

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