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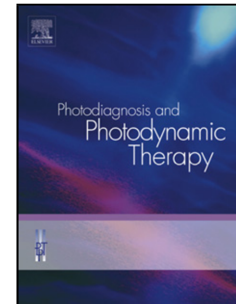

Epithelial tissue thickness improves optical coherence tomography's ability in detecting oral cancer

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Epithelial tissue thickness improves optical coherence tomography's ability in detecting oral cancer

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Highlights

- Oral epithelium measurements made with OCT were valid and reproducible with minor underestimation.
- Epithelial thickness, combined with architectural changes, led to high accuracy in identifying oral cancer in resected margins.
- Future studies on the use of *in vivo* OCT in detection of tissue pathologies in real-time are needed.

Abstract

Background

OCT is a non-invasive imaging technique that enables the measurement of epithelial thickness and architectural changes, which can help in the diagnosis of pre-cancerous and cancerous lesions. The purpose of the study was to assess whether epithelial tissue thickness improves optical coherence tomography's ability in detecting oral cancer.

Patients and Methods

Surgically resected oral margins from 60 patients diagnosed with oral squamous cell carcinoma were subjected to OCT. Three OCT measurements (immediate, 1 hour and 24 hours post-resection) were conducted per resected tissue specimen to look at the effect of saline and formalin on the specimen and its effect on the reproducibility of the OCT. OCT was, then, used to measure the epithelial tissue thickness in cancer-free and cancer-involved margins in eight oral anatomical locations. This data was,

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then, combined with architectural changes data to calculate the sensitivity and specificity.

Results

An overall of 189 cancer-free margins and 51 cancer-involved margins had their epithelial thickness measured using OCT and compared to histopathology. With regards to the validity of the OCT and histopathological measurements, epithelial thickness showed good correlation between different readings at all oral sites. With regards to the reproducibility of the OCT measurements, the mean epithelial thickness for all measurements at first (immediate) and second (1 hour post-resection – saline preserved) measurements was not significantly different. Underestimation of the epithelial depth in cancer-free margins was 20µm, while in the cancer-involved margins was 10µm. Combining data from architectural changes and epithelial thickness, a sensitivity of 92% and a specificity of 94% was achieved.

Conclusion

Oral epithelium measurements using OCT were valid compared to those made with gold standard pathology. Measurements made using OCT was also reproducible with minor underestimation. Epithelial thickness, combined with architectural changes, led to high accuracy in differentiating between cancer-free and cancer-involved margins.

Key words: Optical coherence tomography; histometric; oral epithelium thickness; validation; reproducibility.

Introduction

Taking samples from tissue (i.e. biopsy or surgical margin) for microscopic interpretation of subcellular and morphological features are the gold standard methods for the diagnosis of any suspicious lesion[1]. Such procedure, in certain cases, can be associated with tumour spillage, infection and haemorrhage[1,2]. To alleviate patients' anxiety from undergoing excessive numbers of biopsies and the waiting times for the results, non-invasive techniques could be employed to achieve this, including optical biopsy or optical mapping of tissue. This technique is the least invasive and can provide real-time results.[1]

Optical coherence tomography (OCT) is an optical technique, which can provide optical signature of tissue and identify architectural changes occurring in benign and malignant lesions. OCT is a non-invasive imaging technique that enables the measurement of epithelial thickness and architectural changes through analysis of data scans.[2] It is, to a certain extent, similar to high-frequency sonography, which has been used previously to evaluate tumor thickness, but it is known to suffer weak resolution when evaluating thin lesions.[3]

Our group have previously looked at the architectural changes in normal vs. abnormal oral tissues using OCT. Correct identification of certain structures, including keratin cell layer, epithelial layer, basement membrane and other

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microanatomical structures with high accuracy led to improved understanding of this optical technique and its application in oral tissues.[4] We continued to build on our previous study and accumulated more data, which helped us to identify a variety of architectural changes in benign and malignant oral tissues with high accuracy in an immediate ex-vivo prospective study. These examined pathologies included hyperkeratosis, mucocels and papillomas as well as dysplasia and invasive oral SCC in 125 patients. Optical coherence tomography achieved a sensitivity of 85% and a specificity of 78% in the assessment of oral potentially malignant and malignant disorders, with overall accuracy of 82% based on architectural changes alone.[5]

Furthermore, we used this technique in the assessment of oral squamous cell carcinoma (OSCC) resection margins where we examined 112 margins in 28 T1/T2 N0M0 OSCC patients. The overall sensitivity and specificity, based on architectural tissue changes alone, was found to be 81.5% and 87%, respectively.[6] In this study we identified epithelial tissue thickness as a possible predictor of abnormal tissue changes which can improve OCT accuracy in detecting abnormal pathology.

It is widely accepted that changes to epithelial tissue is related to increased rate of turnover, which are seen in premalignant and malignant lesions.[7,8] In order to map changes affecting the oral epithelial tissue during malignant transformation, we thought it is important to quantify normal epithelial thickness and compare it to suspicious lesions within the same specimen to see if this factor could be used as an aide in improving detection or diagnosis. The purpose of the study was to assess whether epithelial tissue thickness improves optical coherence tomography's ability in detecting oral cancer.

Patients and methods

The immediate ex-vivo prospective study was conducted at University College London. The study was approved by the Ethics Committee of Moorfields & Whittington Local Research Ethics Committee for Human Research according to the principles of the Declaration of Helsinki. Inclusion criteria involved T1/T2 N0 patients with oral squamous cell carcinoma (OSCC) who were treated with tumour resection with/out neck dissection. T3/T4 disease patients were not included in this study due to the difficulty of conducting margin analysis. Any of the patients recruited should not have received radiotherapy or chemotherapy before due to their possible effects on epithelial thickness.

Sixty patients were recruited for the study, agreed to participate and signed an informed consent form. The main data of the study were clinic-pathological parameters, which included scanning resected tissues with OCT and comparing them to the co-localised histopathological slides. An incisional surgical biopsy was acquired from each patient and confirmed the diagnoses of oral squamous cell carcinoma (OSCC). All patients were discussed at our multi-disciplinary meeting.

All surgical resections were performed under general anaesthesia. The resections were transferred to the lab and were subjected to optical coherence tomography.

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We used a swept-source frequency-domain optical coherence tomography microscope (Michelson Diagnostics EX1301 OCT Microscope V1.0), the components of which are illustrated in studies by our group.[4,5,6] The light source used is a Santec HSL-2000, with an imaging wavelength of 1310nm, axial optical resolution of <10µm, and lateral optical resolution of <10µm. The system provides an image resolution of 5.3µm/pixel with a maximum image width of 6mm, a sub-surface imaging depth of 1.5mm, and a focal depth of 1mm. Samples can be manipulated to see the full quality results on the screen instantly, with an image capture time of <100ms and refresh rate of >1Hz (Figure 1).

The OCT scanning was carried out in the immediate ex-vivo phase, then the specimen was preserved in saline and a second OCT scan was conducted at 1 hour post-resection, then the specimen was preserved in formalin and a third OCT measurement was conducted at 24 hours post-resection (Figure 1). The three OCT measurements (immediate, 1-hour and 24-hours post-resection) were conducted to look at the effect of saline and formalin on the specimen, which could potentially affect the reproducibility of the OCT images. The oral epithelium OCT measurement was conducted from the surface to the basement membrane at three predetermined points (distal, middle and medial part of the free margin). The average thickness of the epithelium was calculated from the reading of the best correlation for use in further analysis. This correlation was, then, used to validate the OCT measurements.

The process of co-localisation was described in previous studies published by our group.[4-6] The co-registration process involved diagrams, digital images and specimen orientation using sutures and special ink. For each surgical specimen, OCT images were acquired from the four-resection margins (superior, inferior, medial and lateral). Mean values of a number of metric readings were recorded for the epithelial layers at the resection margins area.

All specimens were then processed for assessment of the surgical margins status. Histopathological assessment was carried out by one oral and maxillofacial pathologist to ensure objectivity. Epithelium thickness was measured by the standard method using an ocular micrometer. The vertical distance from the uppermost level of the squamous cell layer to the lowest point of the basement membrane was recorded in micrometer units. The examiner who measured the epithelium thickness did not know the thickness of the lesion estimated by OCT. The OCT measured epithelium thickness (at 1-hour and 24-hours) was, then, compared with the histopathology slides, and the correlation was statistically evaluated with Pearson's correlation coefficient.

Validity of OCT measurements were determined by comparing the average of the epithelial measurements obtained from histological slides with the average of the OCT measurements. The means of both measurements were compared using a two-tailed paired t-test and the difference between the means was compared to zero. Similarly, reproducibility of the OCT was determined by comparing the average of epithelial thicknesses at immediate post-resection to the average measurements at 1hr post resection. The degree of reproducibility is expressed by the 95% limits of

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agreement (mean ± [1.96 × standard deviation]). The difference between the validity and reproducibility is that the former used gold standard measurement as reference, while the later use another measurement, which is not standard (Figure 2).

A “pre-made proforma” was used to collect clinic-pathological and optical data from each patient included in this study. We use this electronic form in our head and neck unit mainly for audit and research purposes. It includes several sections: general demographics, clinical, pathological, radiology and research data. The proforma has been designed according to the clinical protocols at University College London and defined to maintain patient confidentiality. The researchers were very accurate in record keeping which later on facilitated statistical analysis and study results.

Results

A total of 60 primary oral cancers lesions with 240 resected margins were subjected to OCT. 189 resected margins were cancer-free and 51 tumour-involved margins were subjected to OCT. Forty patients were males (66.6%) and twenty were females (33.3%); with a mean age of 63 years (range 40-97 years). Half of the patients were current smokers. Over one third of the patients consumed alcohol on a regular basis and none of the patients chewed betel nut (Tables 1 and 2).

More than two thirds of the lesions were ulcers; and the rest manifested as plaques or papules. More than one third of the lesions manifested as erythroplakia, the rest presented as leukoerythroplakia and homogeneous leukoplakia. The anatomical distribution of the lesions showed 15 in the lateral border of the tongue, 13 in the floor of mouth, 8 in the ventral tongue and 6 in the buccal mucosa. Clinical staging at time of presentation showed that 43 patients had T_1N_0 disease and 17 patients had T_2N_0 disease (Table 1).

With regards to the validity of the OCT and histopathological measurements, epithelial thickness showed good correlation between different readings at all oral sites. The highest correlation was at 24 hours post post-resection (r=0.964) compared to the first (immediate) and the second (1hr post-resection) readings (r =0.948, r =0.932 respectively), (Figures 3). In the alveolar mucosa, the correlation was (r =0.951; P >0.01). In the buccal mucosa resection margins, OCT and histopathological measurements showed much better correlation (r =0.971), compared to other anatomic sites. Floor of mouth mucosal samples were tomographically and histologically well correlated (r =0.903). The correlation between the OCT and histological measurements of the soft palate, lateral tongue and ventral tongue was excellent (r =0.982, r =0.966 and r =0.987, respectively). The correlation for hard palate was r =0.895. The lowest correlation was for the lower lip (r =0.578).

With regards to the reproducibility of the OCT measurements, the mean epithelial thickness for all measurements at first (immediate) and second (1-hour post-resection [saline preserved]) measurements were not significantly different (t = 2.297, P > 0.05, with a confidence of interval of -0.784 to 1.048). No comparison was

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made to the third measurement (24-hours post-resection [formalin preserved]) as reproducibility results will be significant due to the effect of formalin on the tissue structures, which can cause up to 40% shrinkage in the specimen. The best correlations obtained was at 24-hours post-resection, were used to calculate the average thickness for different types of oral epithelium. The mean epithelial thickness of the cancer-free margins was 320µm, while the cancer involved margins was 580µm (table 3).

In table 4, oral epithelial thickness in cancer-free specimens measured by OCT vs. histology is reported. The maximum underestimation of the thickness using OCT is reported to be 50µm, while the maximum overestimation to be 20µm. Recalibration of the whole data suggests that OCT is likely to underestimate the epithelial depth in cancer-free margins by a mean of 20µm. Sub-group analysis, suggested that the mean underestimation of the buccal mucosa to be 40µm, ventral tongue 40µm and floor of mouth 30µm. Table 4, also reports the minimum and maximal epithelial thickness at 8 oral cancer-free anatomical sites with minimal thickness in lower lip mucosa (10 µm), and maximal thickness reported in the buccal mucosa (500 µm).

Furthermore, the oral epithelial thickness in cancer-involved specimen measured by OCT vs. histology has been examined. The maximum underestimation of the thickness using OCT is reported to be 30µm, while the maximum overestimation to be 10µm. Recalibration of the whole data suggests that OCT is likely to underestimate the epithelial depth in cancer-involved margins by a mean of 10µm. Sub-group analysis, suggested that the mean underestimation of the buccal mucosa to be 20µm and lower lip mucosa 20µm. Table 4, also reports the minimum and maximal epithelial thickness at 8 oral cancer-involved anatomical sites with minimal thickness in lower lip mucosa (130 µm), and maximal thickness reported in the buccal mucosa (900 µm).

Figure 4 illustrates the combined OCT and histopathology from cancer-free and cancer-involved margins. Here small margin of overlap between epithelial thickness measurements can be seen in buccal and alveolar mucosa, with underestimation by OCT is more noticeable in cancer-free margins.

The calculation of the accuracy of OCT has been a challenge as we were dealing with continuous data. These data were combined and then sub classified into units so they could be analyzed as qualitative data. This was achieved by identifying a cut off upper and lower margins as “safe” (true positive or true negative) and anything beyond to represent a “risk” (false positive or false negative). This led to a sensitivity of 90.7% and specificity of 89.9%. The PPV and NPP was 83% and 95.8%, respectively.

Furthermore, we have used previously acquired and published data[4-6] (not included in the study) on tissue architectural changes in oral cancer to see if the accuracy of OCT could be improved by combining both data (tissue thickness and architectural changes). These data alone showed that OCT had a sensitivity of 84.5% and specificity of 90%. When combining the data of both parameters, the resultant

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sensitivity and specificity was 92.2% and 94.2%, respectively. The PPV and NPP was 81% and 97.8%, respectively.

Discussion

OCT is an innovative technique, which enables an optical biopsy of epithelial lesions. OCT is the optical equivalent of ultrasound, using light instead of sound, to produce images of tissue. Resolutions up to 1–2µm can be achieved, being 100–250 times higher than high-resolution ultrasound and approaching that of microscopy. Given the match between OCT imaging and histology in epithelial tissues, OCT can play an important role in the diagnosis of pathological lesions and to detect cancer-free or positive margins after resection.[4-6]

To our knowledge, this is the first comparison and quantitative study of OCT versus histopathology from cancer-free and cancer-involved OSCC margins, in which the thickness of epithelium is measured and compared. A remaining major challenge is the potential difference between the OCT scanned and the histopathological sectioned planes. Using our co-localisation technique (highlighted in the patients and methods section), we aimed to minimise the margin of error. However, it may be easy to end up with tangential sections in histopathology, which may lead to inaccurate tissue thickness measurement as well as problems in the identification of architectural changes, which can affect OCT accuracy.

OCT has received renewed interest in recent years since its adaptation for corneal thickness measurement.[9] Its ability to measure epithelial thickness *in vivo* is a major advantage over traditional instruments designed to measure biochemical and morphological changes. However its application in oral tissue has been explored but not for the assessment of oral epithelium thickness.[10-12] At this stage, all the studies published by our group re OCT and mucosal pathologies were immediate *ex vivo*. We are in the process of addressing the challenges that may rise during our *in vivo* OCT trials.

This study examined normal tissue histometrics allowing for a better understanding of the prospective features of tumor-bearing and or dysplasia-bearing sections of oral mucosa.[2] It was speculated that the increased epithelium might reflect the degree of epithelium dysplasia. In previous studies by our group, we reported statistically significant differences in architectural changes of oral epithelial tissues of dysplastic lesions, benign hyperplastic and/or hyperkeratotic nature when compared with normal counterpart using OCT. This refutes the assumption that hyperkeratosis or hyperplasia may give a false sense of dysplasia using this parameter.[5,6]

Kraft *et al.*[13] provided *in vivo* OCT measurements of epithelial thickness of vocal cords in laryngeal mucosal lesions without measuring the thickness of the normal epithelial counterpart. The analysis was restricted to the laryngeal area only, and not the entire upper aeropdigestive tract. Wong *et al.* measured epithelial thickness by OCT, but did not correlate these findings with histology.[14] Using OCT, to look at eight

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different oral mucosal sites was advantageous to our study and did help create a large data bank which could be used as a reference for future studies. Furthermore, we were successful in measuring mucosal sections invaded with SCC from the same site and compared the epithelial thickness.

In the current study, some marked differences in oral epithelium thickness measurements provided by OCT were seen between the three readings. This is most likely attributed to the specimen shrinkage, mostly seen following fixation with formalin, however the specimen shrinkage start almost immediately after removing it from the human body. Optical density and image resolution was similar for all the groups, after 24hrs. Hence, *In vivo* OCT images are likely to be significantly different from immediate *ex vivo* ones.

The reason behind choosing to conduct the first (immediate post-resection) and second measurements (1-hour after preserving in saline) is to measure the method's reproducibility. It has been shown that the reproducibility of coherence interferometry measurement techniques used in this study had significant impact. This finding is consistent with the results from previous studies by our group and suggests that soft tissue shrinkage play a significant role in validation. The insignificant difference in the correlation between the second (1-hour in saline) and third measurements (24-hours after fixation in formalin) may be attributed to the short time delay for examining these specimens.

Gambichler et al.[15] was unsuccessful in validating OCT and to provide strong correlation using *in vivo* measurements in skin. This was mainly attributed to the problem of specimens' shrinkage after resection. One study demonstrated that up to 47.3% collapse of the tissues occur immediately after resection. This shrinkage usually occurs due to internal collagen fiber contraction.[16] This issue has been avoided in our current research by *ex vivo* tissue scanning which showed maximum tissue stability after the major shrinkage following resection. Kaiser et al. encountered problem with direct probe contact with the tissue under *in vivo* investigation. Even slight pressures created by the probe may be sufficient to alter the measurements, and it is impossible to gauge the amplitude of pressures exerted by the hand-held probe.[17] Our future *in vivo* studies will be looking into the difficulty of validation.

In our study, we reported underestimation of epithelial thickness by OCT to be a factor that is worth studying. This was mainly in cancer-free margins. One hypothesis that might explain this result is that the contraction index of non-keratinized tissue like the buccal mucosa, lip and floor of mouth was slightly greater than the contraction index of the tongue, palate, tongue and gingivae. Another possible reason is that OCT measurements might have not been co-localised perfectly with the histopathology section resulting in a slight error in the thickness measurement. This difference can be magnified if the histological sections are in any way oblique as it is sometimes difficult to orientate formalin fixed tissue so that it is cut perpendicular to the surface.

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The use of histological slides examined with light microscopy for measuring normal and pathological epithelial thickness is not without its disadvantages. The morphometry of histopathological specimens will always be subject to highly variable shrinkage artifacts, and these can vary considerably depending on the nature of the tissue. The present study used immediate *ex vivo* specimens, so that one-third shrinkage of the tissue after resection was avoided. However, further processing of histologic sections may result in additional 20% reduction in size and this was previously reported.[18]

Within the oral cavity and oropharynx, few studies have evaluated healthy epithelium thickness using light microscopy alone. Klein-Szanto and Schroeder demonstrate that oral epithelium varies from 75 to 550µm in thickness. The epithelium of the floor of the mouth was thin (86 ±13µm). The epithelium of the alveolar mucosa was thicker (260 ±40µm) than that of the floor of the mouth. The buccal epithelium, on average, was 480 ±90µm thick and hard palate 248 ±37µm, while the attached gingiva was 255 ±57µm. While the underlying the LP may extend up to 2000µm.[19,20]

The limitation of the current study design is the *ex vivo* manner. Possible error in the use of *ex vivo* OCT concerns variability of the refractive index. This is relevant to our study in a variety ways. Surgical resection affects the hydration level of the tissue by significant decreased tissue perfusion. This would result in an increased refractive index. Underestimating the refractive index would result in an artificially thinned epithelium as measured by OCT and might, in part, explain our data. Despite all this, when combining the architectural changes seen within our specimens as well as epithelial thickness, we could obtain a relatively high sensitivity and specificity.

Another limitation of the current study is that performing measurements obliquely may yield higher or lower values. This error could be minimized with the OCT by capturing and measuring the axial dimension of the thickest part of mucosa. In contrast, some of the oblique measurement is difficult to minimize with OCT because there is no way to ensure that the thickest portion mucosa is consistently measured.

Conclusion

Oral epithelium measurements using OCT were valid compared to those made with gold standard histopathology. Measurements made using OCT was also reproducible with minor underestimation. Epithelial thickness, combined with architectural changes, led to high accuracy in differentiating between cancer-free and cancer-involved margins. This study paves the way to out future studies on the use of *in vivo* OCT in detection of tissue pathologies in real-time.

Competing interests

Mr Colin Hopper is advisory board member at Michelson Diagnostics, Kent, UK.

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Figure 1: A: lateral tongue SCC prior to resection, B: sutures and dye applied to improve the process of co-localisation, C: specimen scanned with OCT.

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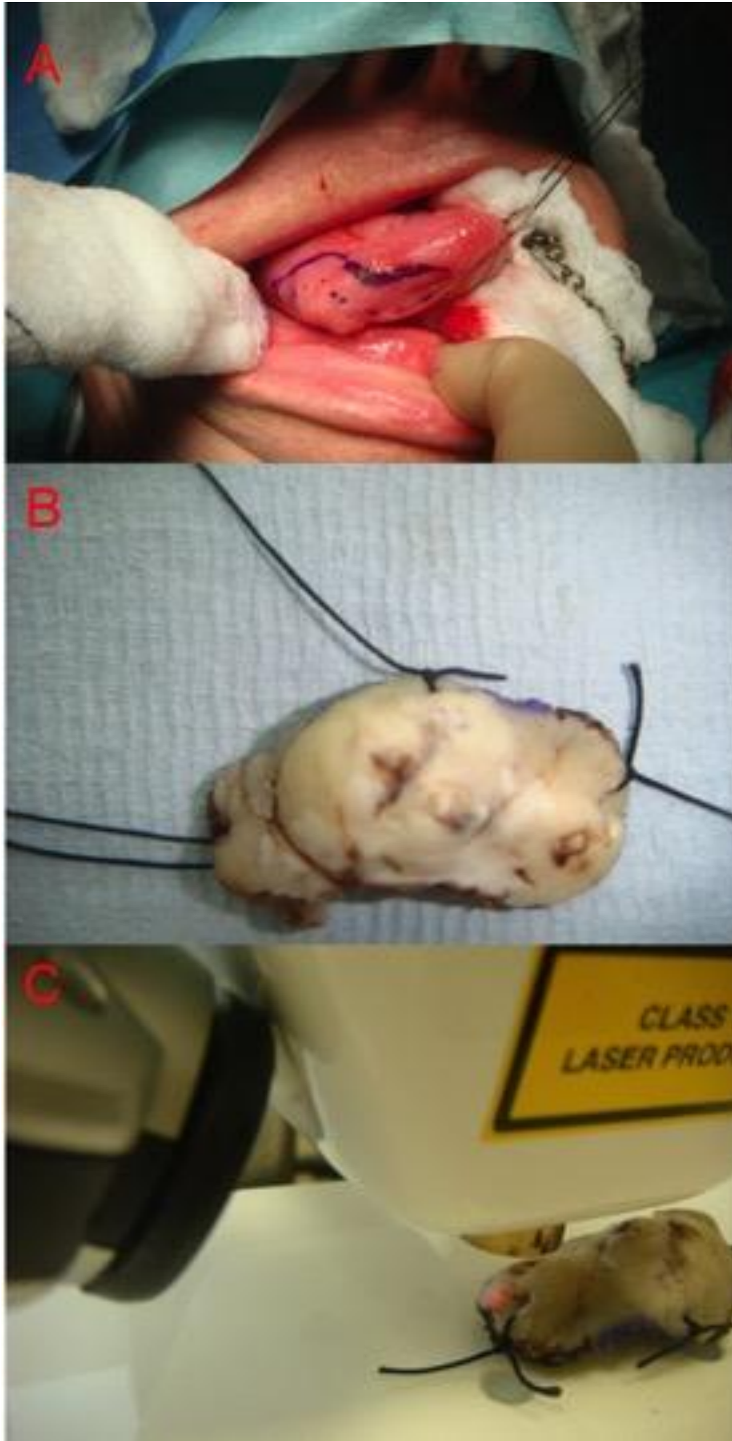

Figure 2: OCT image of oral epithelium used for histometric measurement.

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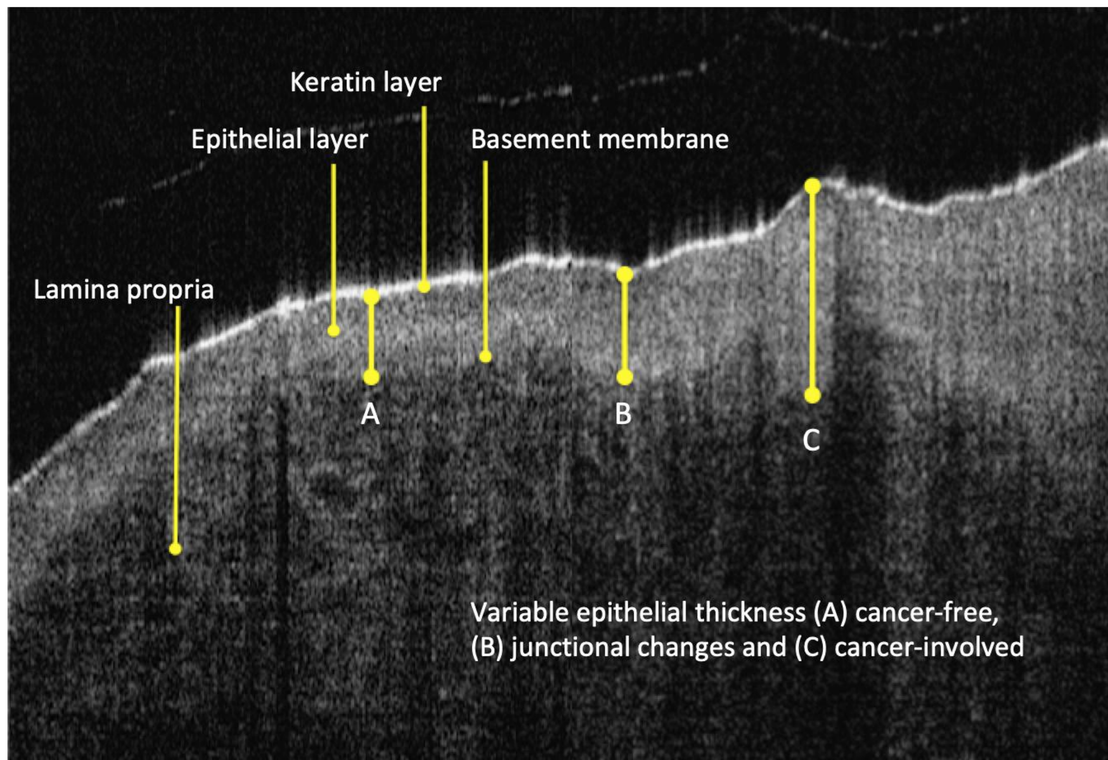

Figure 3: Correlation between OCT and histopathological epithelium thickness in the immediate post-resection phase, at 1-hour (saline preserved) and 24 hours (formalin fixed).

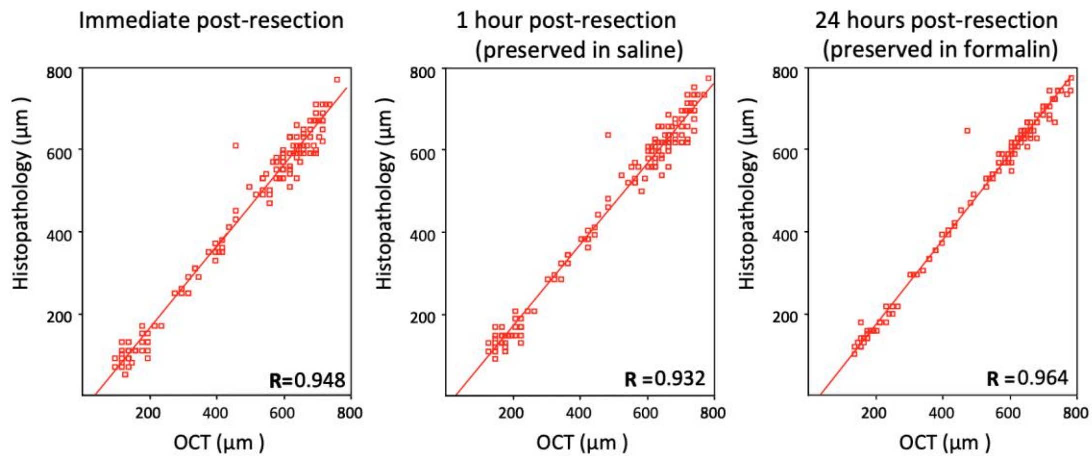

Figure 4: Combined OCT and histopathology from cancer-free and cancer involved margins: epithelial thickness and underestimation.

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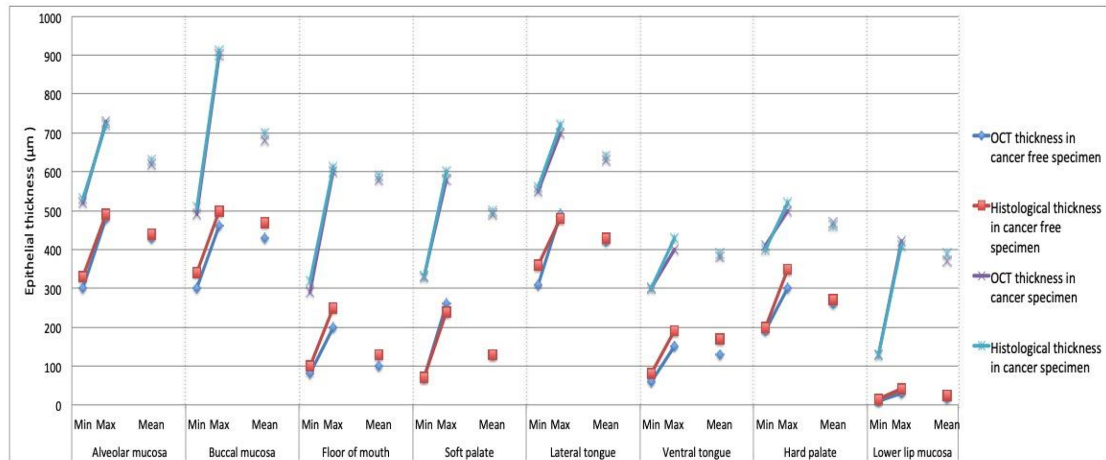

Table 1: Demographics of the cohort included in this study.

	No.		No.
Gender		**Symptoms**	
Male	40	Asymptomatic	46
Female	20	Pain	6
		Bleeding	8
Location			
Lateral tongue	15	**Smoking status**	
Floor of mouth	13	Current smoker	30
Ventral tongue	8	Ex-smoker	18
Buccal mucosa	6	Non-smoker	12
Lower lip	5		
Hard palate	5	**Drinking status**	
Soft palate	4	Current drinker	23
Alveolar mucosa	4	Ex-drinker	12
		Non-drinker	25
Colour			
Leukoplakia	18	**Pan chewing**	0
Erythroplakia	23		
Speckled leukoplakia	19	**Diagnosis**	
		T1 disease	43
Clinical features		T2 disease	17
Plague	6		
Papule	12	**Resection**	
Ulcer	42	Surgical	60
Medical history			
ASA I	50		
ASA II	10		

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Table 2: Histopathological status of the four resection margins of the cohort.

	No.
Superior margin	
Free	50
Involved	10
Inferior margin	
Free	47
Involved	13
Medial margin	
Free	45
Involved	15
Lateral margin	
Free	47
Involved	13

Table 3: Measurement of resection margins using OCT.

	Minimum (µm)	Maximum (µm)	Mean (µm)
Cancer-free margins			
EL thickness	10	500	320
Cancer- involved margins			
EL thickness	130	900	580

Table 4: Oral epithelium thickness in cancer-free and cancer-involved- specimens: OCT vs. histopathology.

	Cancer-free specimens			**Cancer-involved specimens**		
Anatomical area	**Min.** (µm)	**Max.** (µm)	**Mean** (µm)	**Min.** (µm)	**Max.** (µm)	**Mean** (µm)
Alveolar mucosa						
OCT thickness	300	480	430	520	730	620
Histological thickness	330	490	440	530	720	630
Buccal mucosa						
OCT thickness	300	460	430	490	900	680
Histological thickness	340	500	470	510	910	700
Floor of mouth						

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OCT thickness	80	200	100	290	600	580
Histological thickness	100	250	130	320	610	590
Soft palate						
OCT thickness	70	260	130	330	580	490
Histological thickness	70	240	130	330	600	500
Lateral tongue						
OCT thickness	310	490	420	550	700	630
Histological thickness	360	480	430	560	720	640
Ventral tongue						
OCT thickness	60	150	130	300	400	380
Histological thickness	80	190	170	300	430	390
Hard palate						
OCT thickness	190	300	260	410	500	470
Histological thickness	200	350	270	400	520	460
Lower lip mucosa						
OCT thickness	10	30	18	130	420	370
Histological thickness	15	40	25	130	410	390

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