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Optical Coherence Tomography Imaging of Oral Benign Lesions and Comparison of Histopathological Examination

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SUMMARY

Background/Aim: *Optical coherence tomography (OCT) is an optical imaging method used in the examination of superficial tissues in ophthalmology, cardiology, dermatology and dentistry. It can provide high resolution microscopic images and uses infrared light. OCT was used as an auxiliary diagnostic imaging method in this study.* ***Material and Methods:*** *Ex vivo samples of arteriovenous malformation, odontogenic keratocyst, peripheral giant cell granuloma, pyogenic granuloma, irritation fibroma were evaluated with Swept-source OCT. The following features of altered layers were identified from OCT images: (1) irregularity, (2) fragmentation, (3) rupture, (4) interruption, (5) depression, (6) elevation, (7) thinning, (8) thickening, (9) homogeneity (10) hyperreflectivity, (11) continuity of the layers. 3D images were observed both horizontal and vertical planes. Histopathological features were compared.* ***Results:*** *The OCT examination of the tissues allowed to establish clear identification of the stratified squamous epithelium, lamina propria (LP) and basement membrane boundary. The darker appearance of the epithelium is directly related to its lower optical density and scattering properties, which, in turn, result in lower signal intensity. Lamina propria was observed as an hyperreflective layer and appears brighter. Continuity of the layers were well observed.* ***Conclusions:*** *OCT is a non-invasive and promising modality which can be used as an additional method to evaluate the characteristics of surface layers and dysplastic features of the intraoral lesions.*

Keywords: Arteriovenous Malformation, Odontogenic Keratocyst, Optic Coherence Tomography, Peripheral Giant Cell Granuloma

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Introduction

Optical coherence tomography (OCT), first developed by Fujimoto in 1991, is a promising high resolution optical imaging method[1]. OCT uses low-coherence broadband near-infrared light which makes it a non-invasive, harmless imaging method due to the absence of ionizing radiation. According to this principle, OCT gets an excellent spatial resolution (~ 20 μm)[2]. Additionally, concept of OCT is similar with ultrasound scanning except that light is used instead of sound. Besides, OCT is considered superior as it has 10 times higher axial resolution than ultrasound. Surface tissue images are produced by light waves and images constructed in real time and almost similar tissue depth as histological examination. This provides *in vivo* or *ex vivo* imaging of the microscopic characteristics of surface structure which consists of epithelial and subepithelial layers[3].

OCT a common interferometric technique in ophthalmology[4], dermatology[5] and cardiology[6] as it helps to visualize tissue optical properties[7]. New technological improvements provide higher diagnostic accuracy as some of them also provide immediate results. These optical techniques are based on autofluorescence or spectroscopy. Nevertheless, low signal-to-noise ratio is a

limitation for some of these approaches. Although, optical diagnostic approaches have several benefits such as non-invasiveness, absence of ionizing radiation, patient-friendliness, real-time information, repeatability, and high-resolution surface and subsurface images[8].

Optical diagnostic systems avaible in oral and maxillofacial region are tissue auto-fluorescence imaging (VELscope)[9], tissue reflectance (ViziLite)[10], optical biopsy (narrow band imaging)[11], micro-Raman spectroscopy[12], optical coherence microscopy (OCM)[13] and optical coherence tomography (OCT)[3]. Thus, there is an increasing interest in the use of optical diagnostic systems in dentistry. Currently, the use of OCT in dentistry has focused on examining hard tissue[14], enamel cracks and defects can be seen with optical systems[15]. Effective usage of OCT involves examination of changes due to remineralization[15], determining enamel cracks and occlusal carries[16]. Addition to these, the OCT image of healthy oral mucosa allows differentiation from epithelial hyperplasia, mild dysplasia and squamous cell carcinoma. OCT allows the identification of epithelial layer (EL), lamina propria (LP), basement membrane (BM) relations. Epithelium appears darker due to the lower signal intensity. Thus, as an optically dense tissue, LP appears brighter compared to epithelium because of LP's higher intensity[17]. In the light of this information, filiform papillae along the dorsum of the tongue, seromucous glands of lower lip, most superficial muscle layers, oral cancer differentiation are also can be viewed by OCT[18-20]. Additionally, epithelium becomes thicker in non-healthy oral mucosa samples[21]. These features of oral lesions can be examined intraorally or with *ex vivo* samples with OCT.

This study aims to determine superficial layers of oral benign lesions with optical coherence tomography (OCT) and to compare the imaging features with histopathological findings. Surface structure of oral biopsies compared with healthy oral mucosa to assess the disorder.

Material and Methods

Ethical approval for this retrospective study was granted by the Marmara University, Faculty of Medicine, Non-invasive Clinical Research Ethics Committee with the following project no: 09.2021.1246.

Incisional or excisional biopsy was performed from the lesions. *Ex vivo* tissue materials preserved in 10% buffered formalin. In addition, a healthy mucosa sample was excised from buccal mucosa before the extraction of an impacted 3th molar with a view to compare healthy buccal mucosa and alterations of the layers in cases of pathologies.

Biopsy material was evaluated with Optical Coherence Tomography (OCT) (Swept Source, 3D DRI-OCT Triton, Topcon Healthcare Corporation, Japan). This device is an OCT of the type used in ophthalmology and is also considered as an accurate imaging technique for ex vivo oral tissues.

Silicon mould was used to block out peripheral light and to prevent artefacts while scanning *ex vivo* tissues. The silicon material is adapted in the shape of a cone and so that the mould surrounded the lens. This cone, which serves to prevent artefact formation, also eliminated the need for intraoral probe (Figure 1).

In the image obtained with the Macula 3D mode of the OCT device, the hyperreflectivity and continuity of the layers and the surface properties were visualized. Also, lesions were evaluated with the 3D image obtained with the topographic map mode. The tissue was prepared beforehand and fixed with a pin on a foam material with dimensions of 4x4x4 cm.

A total of 10 *ex vivo* tissue samples and 148 optical coherence tomography images of odontogenic keratocyst, arteriovenous malformation, irritation fibroma, giant cell granuloma, pyogenic granuloma and healthy buccal mucosa were evaluated. Healthy oral mucosa sample images were the reference image for monitoring the optical changes of the tissue is the images showing the healthy tissue borders. The following features of altered layers were identified from OCT images (1) irregularity, (2) fragmentation, (3) rupture, (4) interruption, (5) depression, (6) elevation, (7) thinning, (8) thickening, (9) homogeneity, (10) hyperreflectivity, (11) continuity of the layers. Irregularity, fragmentation, rupture, interruption, homogeneity and hyperreflectivity of the superficial layer (EL) were evaluated qualitatively. Depression, elevation, thinning and thickening and continuity of the layers (EL, LP, BM) were evaluated for all layers. 3D images were observed both horizontal and vertical planes.

Ex vivo tissue samples were examined histopathologically after the evaluation with OCT and the diagnoses were confirmed by histopathological examination. Excised lesions were fixed in 10% buffered formalin, embedded in paraffin, and processed for conventional histopathological examination with standard procedure. The sections were then stained with haematoxylin eosin and evaluated under the light microscope. Histopathological images were compared with the OCT images to assess the degree of clinicopathological correlation.

The collected data from all groups were imported to SPSS for Windows, version 22.0 (SPSS Inc., Chicago, IL, USA). The standard descriptive methods such as the mean, standard deviation, median, frequency, minimum and maximum were applied to determine the characteristics of the sample. The Chi-square test was used to compare the categorical demographic variables among the groups. The correlations between at least two continuous variables were examined using Pearson's correlation coefficient.

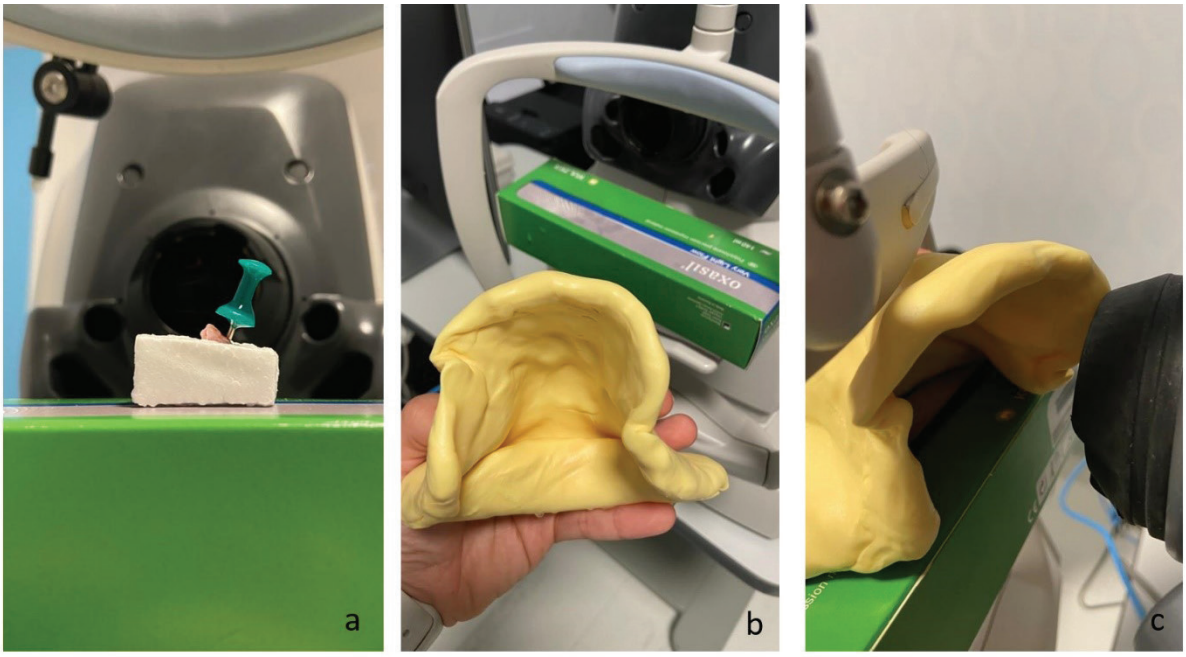

Figure 1. Optical coherence tomography imaging of oral lesions (a) Pinned healthy mucosa sample (b) Silicon mould (c) Covering the lens of the OCT machine with silicon mould to reduce artifacts.

Results

A total of 10 *ex vivo* tissue samples (and 148 OCT images) were evaluated in horizontal and vertical planes. The wavelength was 1,050 nm and the scanning speed was 100.000 A-scan/sec. In depth resolution was between 2.6-8μm while the lateral resolution was 20μm. Displaying format for image evaluation was Macula 3D with 3- 12 mm scanning range in both horizontal and vertical planes.

Evaluated tissue samples were collected from a healthy mucosa (x1), odontogenic keratocyst (x2), giant cell granuloma (x3), arteriovenous malformation (x1), irritation fibroma (x2), pyogenic granuloma (x1).

Irregularity was the most common feature of the benign lesions (95.9%) followed by thinning (91.2%), thickening (89.9%) and continuity of the layers (88.5%). Homogeneity was the least common feature (4.1%). (Table 1)

A statistically significant difference was found between the presence of irregularity and the presence of fragmentation, rupture and interruption in the EL and LP ($p<0.05$). (Table 2, Table 3, Table 4).

Table 1: Frequency of the features.

Presence	Irregularity	Fragmentation	Rupture	Interruption	Depression	Elevation	Thinning	Thickening	Homogeneity	Hyperreflectivity	Continuity of the layers
n	142	63	92	96	127	130	135	133	6	130	131
%	95.9	42.6	62.2	64.9	85.8	87.8	91.2	89.9	4.1	87.8	88.5

Table 2: Relationship between the fragmentation and irregularity

		Fragmentation (n)		**p**
		Presence	Absence	
Irregularity (n)	Presence	63	79	
	Absence	0	6	0.038*

Chi-square test, *=$p<0.05$.

Table 3: Relationship between the rupture and irregularity

		Rupture (n)		**p**
		Presence	Absence	
Irregularity (n)	Presence	92	50	
	Absence	0	6	0.002*

Chi-square test, *=$p<0.05$.

Table 4: Relationship between the interruption and irregularity

		Interruption (n)		**p**
		Presence	Absence	
Irregularity (n)	Presence	96	46	
	Absence	0	6	0.002*

Chi-square test, *=$p<0.05$.

A statistically significant difference was found between the presence of hyperreflectivity and the presence of elevation and depression in the EL and LP ($p<0.05$). (Table 5, Table 6). Similar findings between the OCT and pathological examination were the signal-rich surface layer corresponded

with the thickened epithelium which was a pathological finding. The surface signal-rich layer and the signal-poor areas below corresponded to inner structures of the lesions.

Table 5: Relationship between the elevation and hyperreflectivity

		Elevation (n)		p
		Presence	Absence	
Hyperreflectivity (n)	Presence	117	13	
	Absence	13	5	0.047*

Chi-square test, *=p<0.05.

Table 6: Relationship between the depression and hyperreflectivity

		Depression (n)		p
		Presence	Absence	
Hyperreflectivity (n)	Presence	119	11	
	Absence	8	10	0.000*

Chi-square test, *=p<0.05.

OCT examination of the healthy buccal mucosa allowed identification of the EL along the mucosa surface. In comparison with the upper keratin layer or lower lamina propria, the EL often appeared translucent or less hyperreflective. A lower optical density and scattering properties contribute to the darker appearance of the epithelium. Basement membranes separate LP from EL. Due to the higher signal intensity of the LP, it appears brighter as it has a higher optical density than the LP. Along the BM boundary, the transition between EL and LP tissues occurs. In buccal mucosa epithelium, lower optical density and scattering properties are directly responsible for its darker appearance. As a result, the signal intensity is lower. As a result of its optical density, the LP appears brighter than the BM tissue, due to its higher signal intensity (Figure 2).

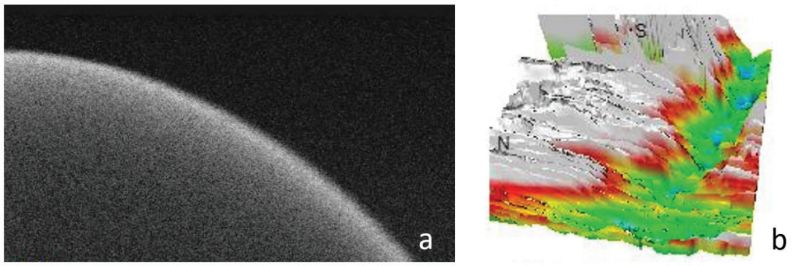

Figure 2. OCT image of the healthy oral mucosa (a) and the surface map (b)

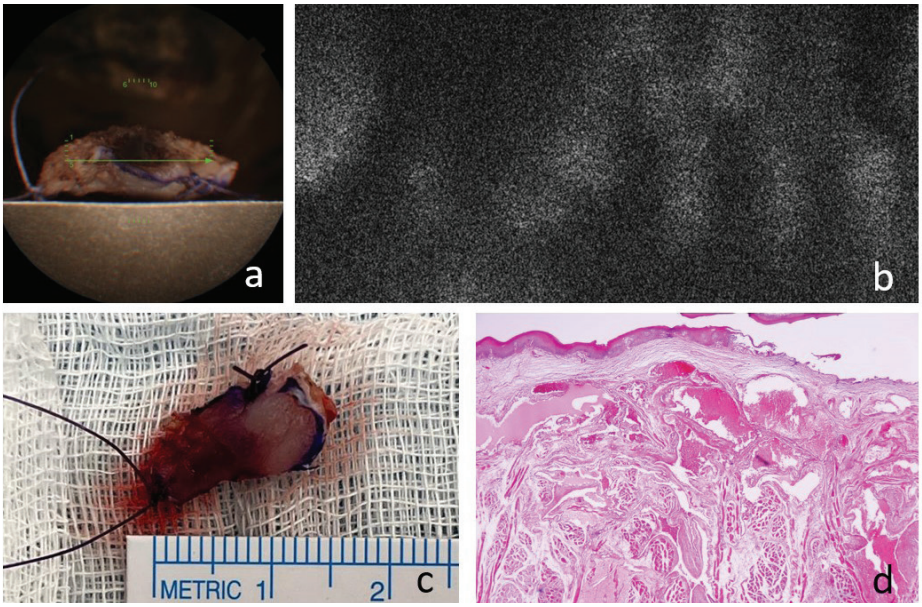

Figure 3. Arteriovenous malformation sample imaging with OCT device (a) OCT image of the lesion with interruption, fragmentation, rupture, irregular surface layer, thinning and thickening observed in axial plane (b) Gross image of the lesion (c) Large vessels extending into the muscle filled with erythrocytes or serum under partially ulcerated epithelium (H&E x100) (d)

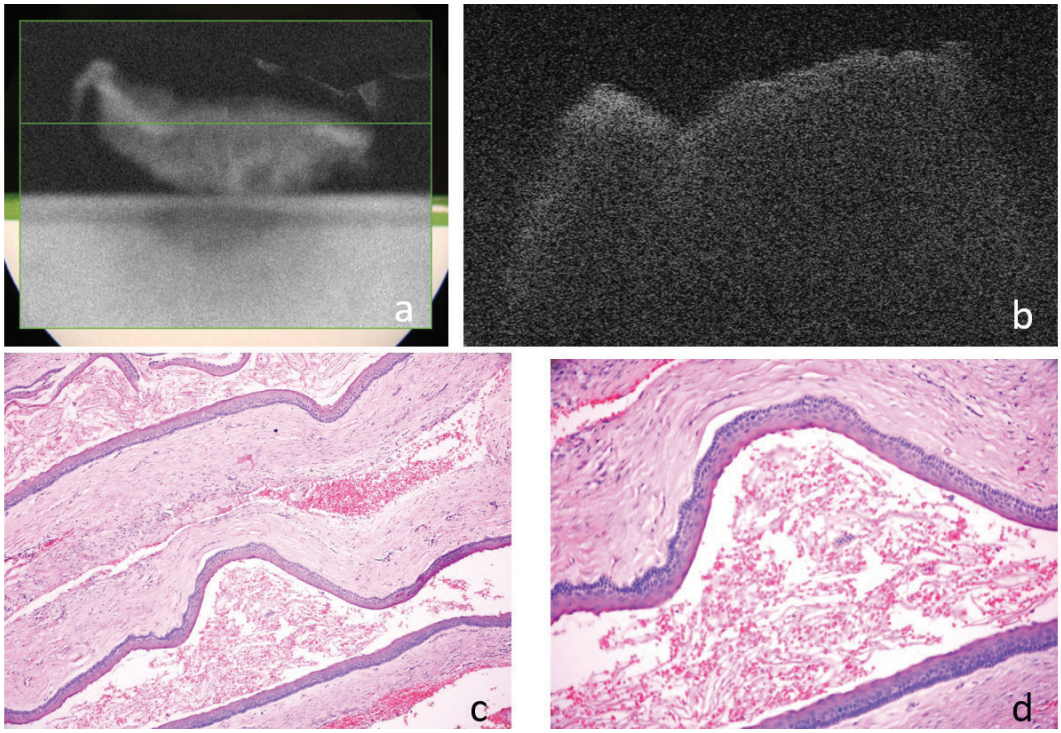

Figure 4. Odontogenic keratocyst sample image with OCT device (a) Thinning and thickening of the irregular surface layer (b) Histopathologically cyst lined by uniformly thin epithelium with abundant keratin in the lumen (H&E x100) (c) Lining epithelium characterized by parakeratosis, surface corrugations, and palisading of basal cell nuclei (H&E x200) (d)

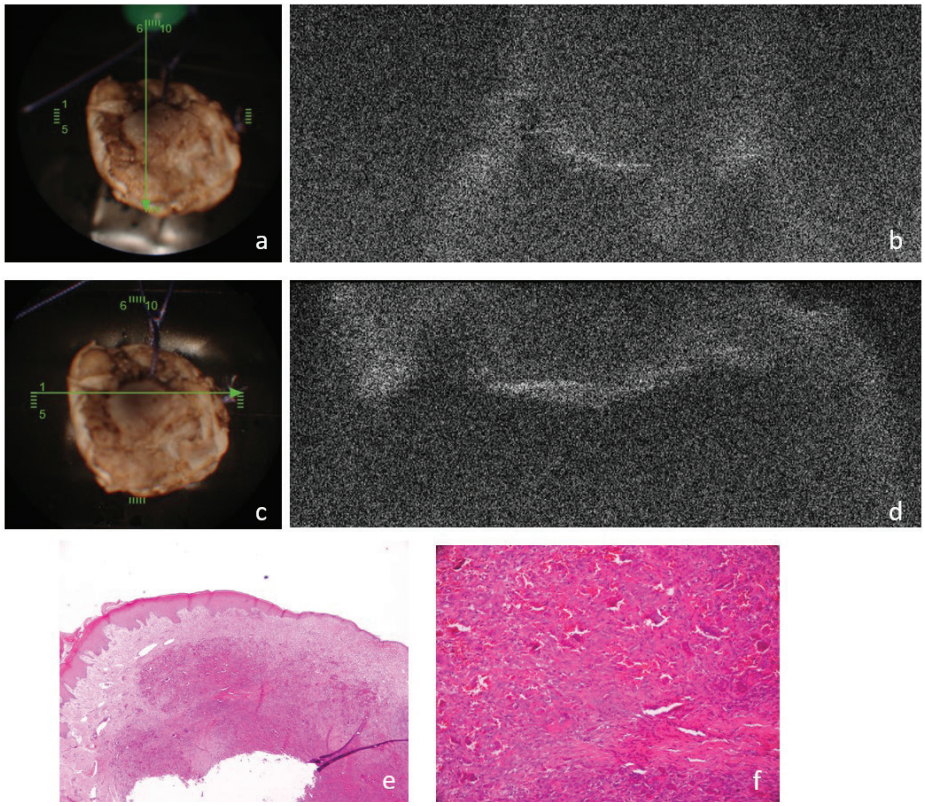

Figure 5. Peripheral giant cell granuloma sample image from OCT device, vertical plane (a) Rupture with thinning and thickening of the surface layer, vertical plane (b) Peripheral giant cell granuloma sample image from OCT device, axial plane (c) Hyperreflective areas are observed irregularly at the surface layer, axial plane (d) The cellular tumor is separated from the epithelium by a clear zone (H&E x100) (e) Sheets of multinucleate giant cells and mononuclear cells in vascular stroma (H&E x200) (f)

Histopathological examination of arteriovenous malformation revealed vessel sections with thick walls which anastomoses with each other were observed. Vessel lumens were filled with erythrocytes and serum were observed under the partly eroded stratified squamous epithelium. Vessel sections intertwined with the muscle tissue was also observed (Figure 3d).

Histopathologically, odontogenic keratocyst samples showed that the cyst epithelium is uniformly 5 to 10 cells thick, with parakeratosis with a corrugated surface. Palisading of the basal cell nuclei, and a flat interface were observed (Figure 4c-d)

Histopathological examination of peripheral giant cell granuloma showed that under the partly ulcerated epithelium, a lobular lesion separated by a clear zone was observed. A vascular-rich focal lesion consisting of proliferation of mononuclear and multinucleate giant cells were determined. Hyperreflective areas represents hyperkeratinization and calcification. GCG images revealed hyperreflective areas that are compatible with histopathological examination (Figure 5).

Pyogenic granuloma, histopathologically demonstrated partly surface ulceration and a subacute inflammatory cell infiltrate comprised of neutrophils, lymphocytes and plasma cell with a lobular arrangement of capillary vessels. OCT images of the pyogenic granuloma showed a regular surface with clear borders. Areas consistent with thinning of the surface layer including hyperreflective areas were visualized.

Histopathologically, irritation fibroma showed a nodular mass composed of fibrous connective tissue with collagen bundles interspersed with fibroblasts, blood vessels under the parakeratinized surface squamous epithelium. OCT images of irritation fibroma and healthy buccal mucosa mostly had similar findings. However, unlike the healthy buccal mucosa, the epithelial tissue was observed as double-layered. This was thought to be compatible with the epithelium of irritation fibroma and the epithelial layer of healthy tissue underneath.

Discussion

Optical coherence tomography (OCT) is a radiation-free, high-resolution method to quantify epithelial thickness in vivo[8-10]. Evaluating structural changes in oral epithelia can help with the detection of oral lesions[3]. Monitoring epithelial changes might also help to improve the detection of oral dysplastic changes and oral cancer treatment[1,4] and manage therapeutic side effects, such as mucositis[5-7]. A good correlation has been confirmed between OCT and histopathological measurements of epithelial thickness[11]. OCT enables non-invasive imaging of multiple sections and can thus be considered an optical biopsy[12]. Currently, two-dimensional (2D) OCT cross-sections are measured at several sites in multiple sections to gain a representative overview of epithelial thickness[4,13].

In the oral cavity, the mucosa is composed of a stratified squamous epithelium overlaid by connective tissue and submucosa. Several malignant and potentially malignant disorders can develop in the tissue, along with alterations in its subsurface microarchitecture. Detecting suspicious lesions early and differentiating them from normal mucosa are critical to achieving an effective treatment[3,17,19]. There are OCT studies that evaluated oral lesions *in vivo* and *ex vivo*, examining oral soft tissue structures.

Thus, there is still not sufficient evidence in terms of imaging oral benign lesions with OCT. In this study, we aimed to evaluate the correlation between optical changes and oral benign lesion characteristics by imaging the surface structures with OCT and to compare with histopathological examination. Also, various oral benign lesion images were evaluated to visualize the benign tissue characteristics. This might improve the differentiation of the oral benign lesions from oral cancers.

As benign tumour of oral mucosa, haemangioma is a term used to describe a variety of developmental vascular anomalies. Vascular malformations are structural anomalies of blood vessels with normal endothelial turn-over[22,23]. In a case analysis of Ozawa et al. two haemangiomas were examined with OCT which was found highly correlated with histopathological findings[22]. Arteriovenous malformation images showed that even though the incidence of thickening, thinning, fragmentation, heterogeneity, hyperreflectivity was significantly high, the continuity of the layers was well observed. Arteriovenous malformations may also show optical changes in EL, LP and BM individually. Additionally, the lesion excised from tongue, so the irregular tongua papillae formation around the lesion was observed in the OCT images.

Odontogenic keratocyst (OKC) is a unique form of developmental odontogenic cyst. Biological behaviour of OKC deserves special consideration due to the high risk of recurrence[23]. To prevent recurrence careful curettage or special treatment of the cyst cavity is necessary as they may contain daughter cysts and epithelial islands in the cyst wall[24]. Irregular hyperreflective superficial layer was found as a common finding in this study. The continuity of the layers was observed well.

Peripheral giant cell granuloma is considered to be a reactive lesion of oral mucosa rather than a true neoplasm. It is commonly caused by local irritation or trauma. Proliferation of multinucleated giant cells and haemorrhage is a characteristic feature of the lesion which also the reason of hemosiderin pigment deposition. Ulceration of the overlying mucosa is not a rare finding. Areas of bone formation or dystrophic calcification are also common with PGCG[23,25]. No previous studies were

found about neither odontogenic keratocyst (OKC) nor peripheral giant cell granuloma (PGCG) OCT imaging. This study presents the *ex vivo* SS-OCT images of oral lesions using OCT scanner. Superficial layer of the lesion was irregular and both elevation and depression were found in OCT images. Irregular hyperreflectivity was found common.

Healthy oral mucosa sample revealed homogenous LP. Although, one of the images contained heterogenous hyperreflective areas in EL. Hyperkeratinization due to chronic irritation of buccal mucosa may cause hyperreflectivity in keratin layer[26]. Prestin *et al*. revealed that the highest epithelial thickness value was found in buccal mucosa and the hard palate[27]. In this current study, irritation fibroma and healthy oral mucosa revealed similar diagnostic features during the evaluation. The hyperreflectivity and the homogenous thickness was found as a common feature of buccal mucosa OCT images. According to the study of Hamdoon *et al*., poor agreement was seen in the status of the lamina propria is concerning. The alteration of light scattering or decreased light penetration due to the lack of tissue perfusion may be a causative factor in that. In this present study, EL followed well, relation of EL and LP was observed well. Thus, LP and BM relation was unclear, similar with Hamdoon *et al.*[28,29]. A study of Ridgway et al. showed that OCT permits the identification of EL, LP and BM relations. Epithelium appears darker due to the lower signal intensity. Thus, as an optically dense tissue, LP appears brighter compared to epithelium because of LP's higher intensity[17]. The results of the study of Tsai et al. also showed that epithelium becomes thicker in non-healthy oral mucosa samples[21].

The study of Obade *et al.* showed that the basal membrane status was a key parameter in the detection of SCC and for differentiating SCC from oral dysplasia or benign disorders and the degree of EL hyperreflectivity may led the differentiation of benign lesions and other pathologies. Increased epithelial thickness and hyperreflectivity are pathological findings in various conditions. In this study, superficial layers of the lesions were found commonly hyperreflective (87.8%). As it was mentioned in the study of Obade *et al*. EL thickness and hyperreflectivity are not certain diagnostic criteria for benign or malignant oral lesions[3]. Irregular superficial layer was seen in 95.9%. Increased hyperreflectivity of the superficial layer was found in 87.7%. As these findings were found common in malignant lesions in previous studies and benign lesions in this study, determining the malignancy from these features is not reliable[3,21,28,29]. Thus rupture, interruption, fragmentation are relatively rare findings in oral benign lesions and the layer integrity is substantial for pre-diagnosis.

Biological tissue can interfere with the polarisation of the light beam, causing birefringence, diattenuation, and depolarisation which is the basic principle of OCT[29]. Deformities of the excised tissue may cause optic artefacts. Compared with scalpel biopsies, punch biopsies are less likely to produce artefacts such as crush, fragmentation, splits, and haemorrhages[30]. Although, as Obade *et al.* did not prefer punch biopsy in their ex vivo OCT study, we did not opt for punch biopsy either[3].

Disadvantages of oral OCT imaging are user-dependent image quality, long scanning time due to pre-imaging preparations and expensive imaging process. Also, SS-OCT is not an easily accessible device for dental clinician. Limitations of this study must be also discussed. The fixation process of the sample in 10% buffered formalin which may change the shape of the tissue and reduce the image quality. Saline should be used to prevent tissue deterioration. Also, the OCT device was applicable for ophthalmology, not for intraoral purposes. The lack of intraoral probe can also be considered a limitation. Further studies should evaluate these intraoral lesions with an intraoral prob during surgical procedures without formalin fixation.

Conclusions

OCT imaging technique is a radiation-free, high-resolution method to qualify epithelial surface features. It can provide accurate layer identification of oral lesions and further evaluation for the diagnosis and treatment of oral tumours. In this context, OCT can provide valuable diagnostic images to both the clinician and the pathologist. A good correlation has been confirmed between OCT and histopathological examinations of lesion surfaces in multiple sections and can thus be considered an optical biopsy.

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