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Potential of optical coherence tomography for early diagnosis of oral malignancies

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Abstract

With nearly 1,500,000 new patients diagnosed every year in the USA, cancer poses a considerable challenge to healthcare today. Oral cancer is responsible for a sizeable portion of deaths due to cancer, primarily because it is diagnosed at a late stage when the prognosis is poor. Current methods for diagnosing oral cancer need to be augmented by better early detection, monitoring and screening modalities. A new approach is needed that provides real-time, accurate, noninvasive diagnosis. The results of early clinical trials using *in vivo* optical coherence tomography for the diagnosis of oral dysplasia and malignancy are encouraging.

Keywords

Clinical need

According to the American Cancer Society, 1,437,180 patients were diagnosed with cancer in the year 2008, with 35,310 being oral cancers [1]. In the same year, 552,200 people were expected to succumb to cancer in the USA, with 7590 of those from oral cancer alone [1]. Oral cancer is the 8th most common cancer among white males and the 6th most common cancer among black men living in the USA [1]. Approximately 9000 deaths occur as a result of these malignancies [1]. More deadly than breast cancer, cervical cancer and prostate cancer, it has been estimated that oral cancer kills one person, every hour of every day [2,3].

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Accounting for 96% of all oral cancers, squamous cell carcinoma (SCC) is usually preceded by dysplasia presenting as white epithelial lesions on the oral mucosa (leukoplakia). Leukoplakias develop in 1–4% of the population [4]. Malignant transformation, which is quite unpredictable, develops in 1–40% of leukoplakias over 5 years [4]. Dysplastic lesions in the form of erythroplakias (red lesions) carry a 90% risk for malignant conversion [4]. Tumor detection is further complicated by a tendency towards field cancerization, leading to multicentric lesions, which may not all be clinically visible [5]. Current diagnostic techniques require a surgical biopsy of lesions. Benign lesions are often biopsied, reducing patient motivation to agree to further diagnostic biopsies in the future. Conversely, many lesions are only detected by biopsy at an advanced stage, when treatment options and outcome are far from optimal. Of all oral cancer cases documented by the National Cancer Institute (NCI) Surveillance, Epidemiology, and End Results Program, advanced lesions outnumbered localized lesions by more than 2:1. The 5-year survival rate is 75% for those with localized disease at diagnosis, but only 16% for those with metastasis [4,6]. Despite significant advances in cancer treatment, early detection of cancer and its curable precursors remains the best way to ensure patient survival and quality of life.

In anatomical sites, such as the oral cavity, early recognition of malignancy is problematic owing to the frequent lack of gross signs or obvious symptoms. In many cases, detection is further hampered by poor visual access, difficulty in determining which of the encountered dysplastic regions will transform into malignancy and the inability to perform adequate or regularly repeated screening in high-risk patients. A modality for the direct, noninvasive early detection, diagnosis and monitoring of oral dysplasia and malignancy as well as the screening of high-risk populations is urgently required to identify treatment needs at early, more treatable stages of pathological development.

Importance of early detection

The prevention of oral cancer and its associated morbidity and mortality hinges upon the early detection of neoplastic lesions, allowing for histologic evaluation and treatment as necessary. Although basic oral cancer examination to achieve early detection requires only a 90-s visual and tactile examination, too few practitioners, and dentists in particular, are conducting these exams [7]. Moreover, the identification of high-risk individuals would permit the development and implementation of efficient chemoprevention and molecular targeting strategies.

There is a general consensus that the clinical stage at the time of diagnosis is the most important predictor of recurrence and death in head and neck cancer patients. The time of diagnosis is influenced by multiple clinical and sociodemographic variables, including patient reluctance to consult a healthcare professional owing to lack of access that is all too common, especially in patients with low socioeconomic status, as well as professional delay in diagnosing and treating the disease. Studies have demonstrated that dentists and other healthcare providers are in desperate need of systemic educational updates in oral cancer prevention and early detection, as they are remiss in the provision of oral examinations and in the detection of early oral cancers [8]. Clinicians can increase survival rates if a cancerous lesion is detected at an early stage or if a precursor lesion (dysplasia) is discovered and

treated prior to malignant progression [9]. Recent models determining the value of a population-based oral cancer screening program show it to be a promising health promotion strategy (especially in highrisk individuals) with significant increases in quality-adjusted life years saved, which await further economic appraisal [10].

Existing diagnostic tools for oral cancer

Existing clinical diagnostic tools developed for the early detection of oral cancer include tolonium chloride or toluidine blue (TB) dye, Oral CDx® brush biopsy kits, ViziLite®, salivary diagnostics and several imaging devices, such as Velscope® and multispectral optical imaging systems. To date, none have shown equivalency or been confirmed to be superior to clinical examination [11,12].

Visual examination & biopsy

Since 11% of dentists and 45% of physicians do not feel adequately trained to complete an effective oral cancer examination, this results in a failure to examine for oral cancer [13]. The current approach to detecting the transformation of leukoplakia/erythroplakia to SCC is regular surveillance combined with biopsy or surgical excision. However, visual examination provides very poor diagnostic accuracy and biopsy techniques – the current gold standard – are invasive and unsuitable for regular screening of high-risk sectors of the population [1,2,14-17]. Adequate visual identification and biopsy of all such lesions to ensure that they are all recognized and diagnosed is difficult, given the often multifocal nature of oral malignancy [15].

Vital staining

Several studies have investigated the use of vital staining with agents such as Lugol iodine, TB and tolonium chloride for detection of oral malignancy [18-23]. Although the sensitivity of these agents in the hands of experts generally approximates 90%, specificity of these agents is poor; sensitivity rapidly decreases when this modality is used by nonexperts, such as screeners in field units [18-23]. From recent studies a relationship between TB staining and genetic changes associated with the progression of potentially malignant lesions to oral cancer – such as allelic loss or loss of heterozygosity (LOH) – was demonstrated [24]. Furthermore, the authors demonstrated in a longitudinal study that TB identified LOH-positive lesions that subsequently progressed to oral cancer [24].

Chemiluminescence: ViziLite

This noninvasive screening tool consists of an acetic acid wash and a single use 'chemi-light stick' that generates a moderately short wavelength light with peak outputs near 430, 540 and 580 nm for illumination of the oral cavity (ViziLite). It is based on the rationale that the typically greater nuclear content density and mitochondrial matrix of abnormal squamous epithelial cells will reflect light and appear white when viewed under a diffuse low-energy wavelength light. Normal epithelium will absorb the light and appear dark [25]. The majority of studies investigating chemiluminescence evaluate subjective perceptions of characteristics of intraoral lesions, including brightness, sharpness and texture versus routine clinical examination. Results have been contradictory [11,26]. Recently a combination of

both TB and ViziLite systems (ViziLite Plus with TB system) has been introduced. A new chemiluminescence device (MicroLux DL) has also recently been introduced on the market [27].

Oral brush cytology

The brush biopsy (CDx) was designed for use on clinical lesions that would otherwise not be subjected to biopsy because the level of suspicion for carcinoma, based upon clinical features, was low. Using cytological examination of 'brush biopsy' samples as a noninvasive method of oral diagnosis has been shown to provide moderate sensitivity levels of detection of oral epithelial dysplasia or SCC (70–90%) but poor specificity (3–44%). Thus, this approach is of limited diagnostic value without augmentation by traditional biopsy [28-44].

Spectroscopy

Spectroscopy provides information regarding the biochemical composition and/or the structure of the tissue that conveys diagnostic information. Malignancy-related biochemical and morphologic changes perturb tissue absorption, fluorescence and scattering properties [45,46]. Thus, biochemical information can be obtained by measuring absorption/reflectance, fluorescence or Raman scattering signals [45-50]. Structural and morphological information may be obtained by techniques that assess the elastic scattering properties of tissue [49,51-53].

For decades the use of tissue autofluorescence has been described to screen and diagnose precancers and early cancer lesions in organs such as the lung, uterine cervix, skin and, more recently, the oral cavity [49,54-57]. The concept behind tissue autofluorescence is that changes in the structure (e.g., hyperkeratosis, hyperchromatin and increased cellular/nuclear pleomorphism) and metabolism (e.g., concentration of flavin adenine dinucleotide [FAD] and nicotinamide adenine dinucleotide) of the epithelium, as well as changes of the subepithelial stroma (e.g., composition of collagen matrix and elastin), alter their interaction with light. Specifically, these epithelial and stromal changes can alter the distribution of tissue fluorophores and as a consequence the way the tissues fluoresce after stimulation with intense light (typically, blue light excitation at 400–460 nm). The autoflorescence signal can be directly visualized by the clinician [54-58].

One of the tissue fluorescence imaging systems that has been marketed to dental offices is the Velscope system. In the oral cavity, normal oral mucosa emits a pale green autofluorescence when viewed through the instrument handpiece, while abnormal tissue displays a decreased autofluorescence and appears darker with respect to the surrounding healthy tissue [55-57]. Studies have shown that Velscope can improve lesions' contrast and, therefore, improve the clincian's ability to distinguish between mucosal lesions and healthy mucosa [55-57]. In a recent study using 56 patients with oral lesions and 11 normal volunteers, normal tissue could be discriminated from dysplasia and invasive cancer with a 95.9% sensitivity and 96.2% specificity in the training set, and with a 100% sensitivity and 91.4% specificity in the validation set. Disease probability maps qualitatively agreed with

both clinical impression and histology [57]. Further clinical studies are needed in diverse populations to fully evaluate the clinical usefulness of this promising approach.

Structural and morphological information may be obtained by spectroscopic techniques that assess the elastic-scattering properties of tissue [45]. Pursuant to encouraging preliminary data, clinical trials of elastic scattering spectroscopy sometimes in combination with fluorescence spectroscopy or imaging, are underway [53]. Other studies combine spectroscopy with polarized light and/or fluorescence imaging, and/or *in vivo* microscopy. Devices under development and testing include the FastEEM4® System, the Identafi™ and the PS2-oral®. These clinical studies are still at a relatively early stage and preliminary results are encouraging [46-50,54,59-64]. Remicalm's Identafi technology combines anatomical imaging with fluorescence, fiber optics and confocal microscopy with the goal of precisely mapping the location and determining the extent of the disease in the area being screened. A study in 124 subjects determined a sensitivity of 82% and a specificity of 87% for differentiating between neoplastic and non-neoplastic sites in the oral cavity. Results differed between different sampling depths and keratinized versus non-keratinized tissues [63].

Significant challenges to the use of diagnostic spectroscopy include the often low signal-to-noise ratio, difficulty in identifying the precise source of signals, data quantification issues, and establishing definitive diagnostic milestones and end points, especially given the wide range of tissue types contained within the oral cavity. Limited tissue penetration and concerns regarding mutagenicity when using UV light present further clinical challenges. The abundance of data/information generated in association with our incomplete understanding of the carcinogenesis process tend to render data analysis and interpretation very complex, however, the development of diagnostic algorithms may be able to mitigate this challenge [46,63].

In vivo confocal imaging

In vivo confocal imaging resembles histological tissue evaluation, except that 3D subcellular resolution is achieved noninvasively and without stains. In epithelial structures, resolutions of 1 μm have been achieved with a 200–400-μm field of view [65-69]. While this technology can provide detailed images of tissue architecture and cellular morphology, a very small field of view and limited penetration depth of 250–500 μm considerably reduce the clinical usefulness of this approach.

Photosensitizers

Topical or systemic application of photosensitizers can selectively render pathologic tissues fluorescent when exposed to specific wavelengths of light, this technique has extensively been used for skin and esophageal cancer [70,71]. This induced fluorescence can be used to identify and delineate areas of pathology. Although the fluorescence may be strong enough to be detected with the naked eye [72,73], usually some sort of fluorescence detection device is used to enhance fluorescence visualization and assist with accurate lesion mapping. While many agents are under investigation, or in clinical use outside of the USA, the US FDA approval for photo-sensitizing drugs remains limited. Some promising agents for pho-

todetection include aminolevulinic acid (ALA; Levulan®), hexyl aminolevulinate (Hexvix®), methyl aminolevulinate (Metvix®), tetra(meta-hydroxyphenyl)chlorin (mTHPC®), as well as porfimer sodium (Photofrin®) [72-76]. In a blinded clinical study of 20 patients with oral neoplasms, the diagnostic sensitivity using unaided visual fluorescence diagnosis or fluorescence microscopy approximated 93%. Diagnostic specificity was 95% for visual diagnosis, improving to 97% using fluorescence microscopy. The differences between healthy tissue versus dysplasia versus malignancy were all significant (p < 0.05) [73].

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

Optical coherence tomography

Optical coherence tomography (OCT) is an imaging modality capable of providing noninvasive cross-sectional imaging of biological tissue [77-79]. Frequently, OCT is compared with ultrasound imaging because both technologies employ backscattered signals reflected from different layers within the tissue to reconstruct structural images. In contrast to conventional medical imaging modalities, OCT provides images with high resolution (micrometer scale) in real time. These images can be obtained noninvasively *in vivo* by the use of flexible fiberoptic OCT probes. OCT has a wide range of potential applications in diagnosing diseases in various structures, such as the eye, skin, gastrointestinal, respiratory and genitourinary tracts, cardiology and the oral cavity [80-100]. OCT penetration depths in soft tissue are approximately 2–3 mm, enabling high-resolution imaging of the surface and subsurface tissues as well as any structures accessible by endoscopic probes with near histopathological level resolution [101]. This permits *in vivo* noninvasive imaging of the macroscopic characteristics of epithelial and subepithelial structures, including depth and thickness, histopathological appearance and peripheral margins.

Optical coherence tomography was first used to visualize human tissue in 1991. It has been since refined and accepted as a necessary imaging modality in ophthalmology and non-ophthalmologic OCT systems are increasingly becoming available for medical use. Conventional time domain OCT is based on a scanning optical delay line, which limits its imaging speed [102]. A relatively new technology of OCT, Fourier domain OCT, which measures the magnitude and delay of backscattered light by spectral analysis of the interference pattern, can achieve a 50–500-fold increase in imaging speed and a much higher sensitivity compared with the time domain OCT technique [103,104]. Two methods have been developed for the Fourier domain techniques: a spectrometer-based system that uses a high-speed line-scan camera [105,106] and a swept-laser source-based system that uses a fast wavelength scanning laser [107-110]. At the working wavelength of 800 nm for

ophthalmology imaging, the spectrometer-based system is dominant since it can use the economically priced high-speed line-scan cameras and superluminescent diode (SLD) light sources with broad bandwidths for high axial resolutions [105,106]. At the working wavelength of 1310 nm, a customized large-array line-scan camera has to be fabricated for this system, making the swept-source-based approach a better choice for this wavelength region. Swept-source OCT (SSOCT) systems have reduced sensitivity roll-off with imaging depth compared with spectrometer-based systems [104]. In addition, SSOCT has the advantages of a simple system setup and the capability for balanced detection. A narrower spectral line width can also be achieved without crosstalk, resulting in a larger imaging range.

Clinical studies using OCT for oral premalignancy & malignancy

In a recent independent, blinded study, the clinical diagnostic capability of *in vivo* OCT for oral dysplasia and malignancy was investigated in 50 patients with oral leukoplakia or erythroplakia lesions [111]. The OCT image of a dysplastic lesion (Figure 1B) parallels histopathological status (Figure 1C), showing epithelial thickening, loss of stratification in lower epithelial strata, epithelial downgrowth and loss of epithelial stratification as compared with healthy oral mucosa (Figure 1D). Figures 2A & C show clinical appearance and histopathology, respectively, of an area of SCC on the buccal mucosa. In the OCT image (Figure 2B), the epithelium is highly variable in thickness, with areas of erosion and extensive downgrowth and invasion into the subepithelial layers. The basement membrane is not visible as a coherent landmark. Statistical analysis of the data confirmed the capability of *in vivo* OCT for detecting and diagnosing oral premalignancy and malignancy in human subjects, with excellent intraobserver agreement between OCT scores (Cohen's kappa of 0.872), interobserver agreement for OCT (Cohen's kappas of 0.870), agreement between OCT and histopathology (Cohen's kappa of 0.896). For detecting carcinoma *in situ* or SCC versus non-cancer, sensitivity was 93.1% and specificity was 93.1%; for detecting SCC versus all other pathologies, sensitivity was 93.1% and specificity was 97.3%. Anticipated difficulties with movement artifacts were successfully avoided by seating patients in a reclining dental chair with headrest, neck and arm support. OCT imaging was rapid, unproblematic and well-received by all patients, with the imaging protocol adding only a few minutes to visit duration. An added benefit of the imaging procedure was improved patient compliance for necessary biopsy procedures after viewing OCT images. Thus, the introduction of OCT imaging techniques to routine patient visits should be well-received by patients and clinicians alike.

Other studies utilized direct analysis of OCT scan profiles rather than image-based criteria as a means of delineating oral cancer lesions [100,101]. Specifically, the lateral variation of A-scan profiles to demonstrate two parameters of the OCT signal was used. One of the parameters is the decay constant in the exponential fitting of the OCT signal intensity along depth. This decay constant decreases as the A-scan point moves laterally across the margin of a lesion. The other parameter is the standard deviation of the SSOCT signal intensity fluctuation in an A-scan. This parameter increased significantly when the A-scan point was moved across the transition region between the normal and abnormal portions. Such parameters may well be useful for establishing an algorithm for detecting and mapping the

margins of oral cancer lesions. This capability has a huge clinical significance because of the field cancerization effect in the oral cavity and because of the need to better define excisional margins during surgical removal of oral premalignancy and malignancy.

Optical coherence tomography is also under investigation for a host of innovative applications in related anatomical sites, such as the upper GI tract – especially Barrett's esophagus – and in otolaryngology. These topics fall outside the scope of this article, but we encourage readers to peruse some of these outstanding and informative papers.

Expert commentary

The need for improved early detection and diagnosis of oral lesions, and the importance of adequate noninvasive monitoring and screening tools is obvious. Early clinical trials using *in vivo* OCT imaging have demonstrated the potential for effective OCT-based oral diagnosis and for developing a strong diagnostic algorithm for mapping oral lesions based on data from OCT scans. Thus, these studies support the concept that OCT will be a very useful tool for the early detection and diagnosis of oral lesions, as well as regular monitoring of suspect lesions in the oral cavity and rapid, low-cost screening of high-risk populations. Particularly attractive to clinicians are the ease and speed of imaging, the ability to view immediately and at high-resolution the surface and subsurface microanatomy of the tissues, and ease of image interpretation [110]. In its early stages, as OCT is introduced into a wider clinical setting and undergoes further testing and optimization, its initial primary use may be as a screening and diagnostic tool in a single episode of care 'see and treat' protocol for preinvasive oral cancer. As the technology and techniques evolve, this modality should progressively reduce the need for biopsy, define surgical margins and provide a direct evaluation of the effectiveness of therapy.

Five-year view

The potential for OCT-based diagnostics in the oral cavity is excellent. The penetration depth of this modality in oral hard and soft tissues is adequate for most dental applications. Ongoing innovations include 3D imaging, Fourier-domain OCT, permitting up to 100-times faster acquisition of 3D OCT images and spectral OCT, providing enhanced image contrast and permitting spatially-resolved detection and quantification of changes within the tissues. Polarization-sensitive OCT can be used to enhance collagen imaging, and Doppler OCT for adding a vascular component to the imaging data. Improvements in OCT probe technologies, including emerging microelectromechanical system-based techniques, which permit the construction of very small probes with high-resolution suitable for endoscopic use, are significant factors permitting better and faster access to imaging sites. Several manufacturers are working on the development of OCT systems specifically for use in the oral cavity, and over the next 5 years we expect to see several oral OCT devices become available to clinicians.

As OCT resolution capabilities improve, a need has arisen for better contrast levels in the images. Several different approaches to this challenge are currently under investigation. Gold nano-particles are particularly promising *in vivo* OCT contrast agents because they are biocompatible, easy to synthesize and functional with additional modalities. Doubtless, OCT

contrast-enhancing agents that can be used clinically will be a topic of great interest over the next 5 years.

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Key issues

- Oral cancer contributes to a large portion of deaths occurring due to cancer each year. This is primarily due to the fact that most oral cancers are detected late, when treatment options are poor.

- Current methods rely primarily on visual diagnosis and conformational biopsies.

- A new clinical modality that is direct, noninvasive, quick, and cost effective to screen at-risk populations is needed.

- Optical coherence tomography (OCT) has shown promise in early clinical trials to effectively diagnose oral dysplasia and malignancies.

- OCT is easy to use and relatively inexpensive.

- OCT has the potential to be easily integrated into a primary use for oral cancer screenings.

- Means of combining OCT with other diagnostic entities, such as acoustics, nanoparticles or molecular markers, are important concepts for the future.
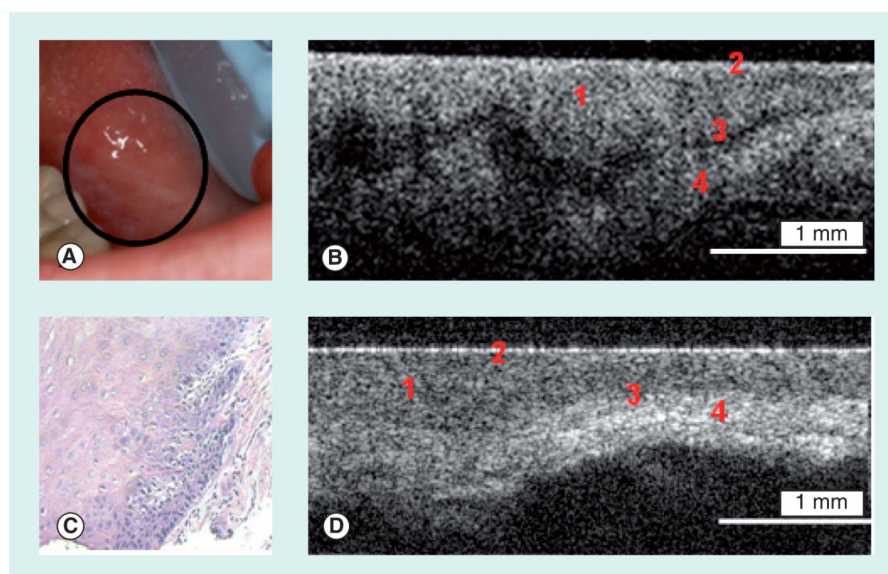
Figure 1. Dysplastic and normal buccal mucosa

(A) Photograph, **(B)** *in vivo* optical coherence tomography image and **(C)** hematoxylin and eosin (10×) of dysplastic buccal mucosa. **(D)** *In vivo* optical coherence tomography image of normal buccal mucosa. 1: Stratified squamous epithelium; 2: Keratinized epithelial surface layer; 3: Basement membrane; 4: Submucosa.

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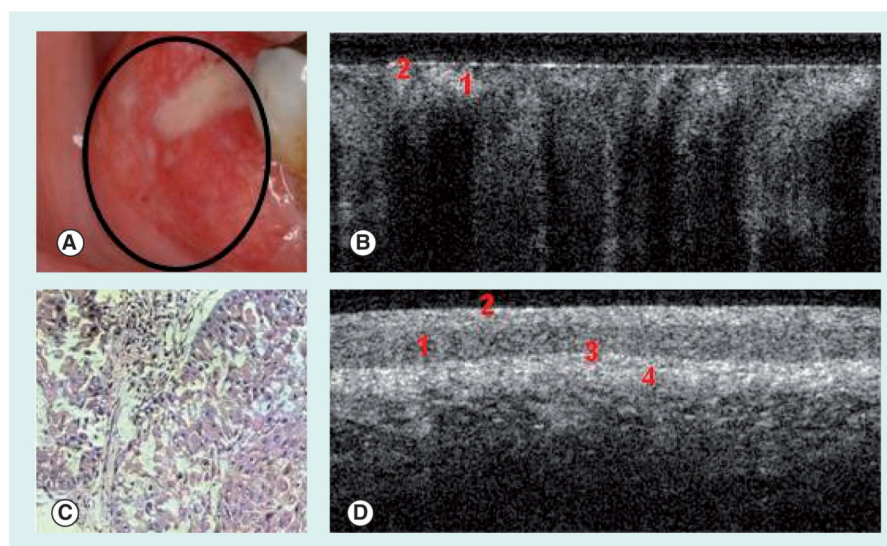
Figure 2. Squamous cell carcinoma of the buccal mucosa

(A) Photograph, **(B)** *in vivo* optical coherence tomography image and **(C)** hematoxylin and eosin (10×) of buccal mucosa with squamous cell carcinoma. **(D)** *In vivo* optical coherence tomography image of normal buccal mucosa.

1: Stratified squamous epithelium; 2: Keratinized epithelial surface layer; 3: Basement membrane; 4: Submucosa.

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