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INVITED MEDICAL REVIEW

Optical diagnostics in the oral cavity: an overview

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As the emphasis shifts from damage mitigation to disease prevention or reversal of early disease in the oral cavity, the need for sensitive and accurate detection and diagnostic tools become more important. Many novel and emergent optical diagnostic modalities for the oral cavity are becoming available to clinicians with a variety of desirable attributes including: (i) non-invasiveness, (ii) absence of ionizing radiation, (iii) patient-friendliness, (iv) real-time information (v) repeatability, and (vi) high-resolution surface and subsurface images. In this article, the principles behind optical diagnostic approaches, their feasibility and applicability for imaging soft and hard tissues, and their potential usefulness as a tool in the diagnosis of oral mucosal lesions, dental pathologies, and other dental applications will be reviewed. The clinical applications of light-based imaging technologies in the oral cavity and of their derivative devices will be discussed to provide the reader with a comprehensive understanding of emergent diagnostic modalities.

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Keywords: optical; diagnosis; oral; dental

Introduction

Light-based imaging of the tissues detects minimal changes such as (i) cell microanatomy, e.g. nuclear/cytoplasmic ratio, (ii) redox status, (iii) expression of specific biomarkers, (iv) tissue architecture and composition, (v) chemical changes (e.g. mineralization), and (vi) vascularity/angiogenesis and perfusion. These properties are ideal for the detection of minimal (early) changes, for assessing the margins of lesions and potentially the presence of subclinical abnormalities beyond the clinical margins, for repeated non-invasive monitoring of existing lesions, and for rapidly examining at-risk populations.

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Oral mucosal lesions

Oral cancer

Chemiluminescence: ViziLite™ (Zila Pharmaceuticals, Inc., Phoenix, AZ, USA.) This imaging device has been FDA-approved for use in the oral cavity since November 2001. After rinsing with an acetic acid mixed solution, the oral cavity is examined under chemiluminescent illumination at 430, 540 and 580 nm wavelengths. This method allows increased visual distinctions between normal mucosa and oral white lesions (Huber *et al*, 2004; Epstein *et al*, 2006, 2008; Kerr *et al*, 2006). The detected signals may be related to the altered thickness of the epithelium, or to the presence of a higher density of nuclear content and mitochondrial matrix that preferentially reflect light. Hyperkeratinized or dysplastic lesions appear distinctly white when viewed under a diffuse low-energy wavelength light. By contrast, normal epithelium will absorb light and appear dark (Lingen *et al*, 2008) (Figure 1). As the majority of studies investigating chemiluminescence reported subjective perceptions of intra-oral lesions in terms of brightness, sharpness and texture *vs* routine clinical examination, data interpretation may vary significantly between examiners (Huber *et al*, 2004; Kerr *et al*, 2006). In January 2005, a combination of both Toluidine Blue and ViziLite™ systems (ViziLite Plus with TBlue system™; Zila Pharmaceuticals, Inc.) received FDA clearance as an adjunct to visual examination of the oral cavity in populations at increased risk for oral cancer. In a multicenter study of high-risk patients, it was reported that the majority of lesions with a histological diagnosis of dysplasia or carcinoma-in-situ were detected and mapped using ViziLite™ and toluidine blue (Epstein *et al*, 2008). Recently, a new chemiluminescence device (MicroLux DL™; Zila Pharmaceuticals, Inc.) has been introduced as an adjunct tool for oral lesion identification (McIntosh *et al*, 2009).

Spectroscopy and autofluorescence Tissue autofluorescence has been applied in the screening and diagnosis of pre-cancer and early cancer of the lung, uterine cervix, skin and, more recently, of the oral cavity. During the

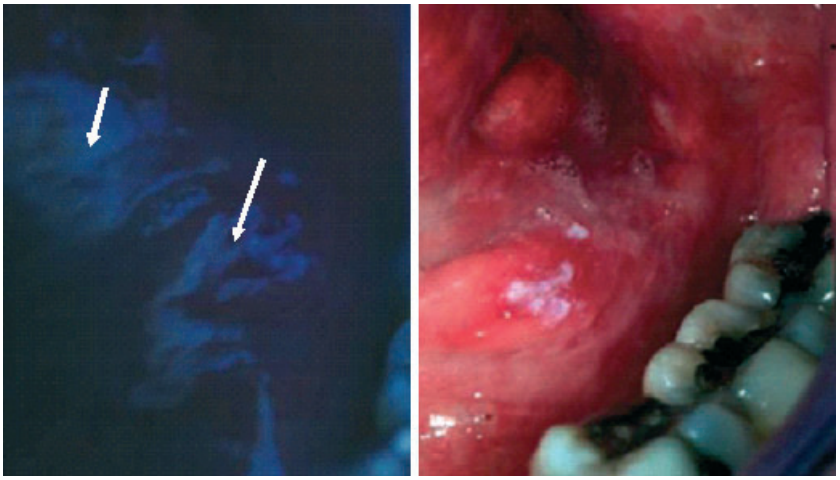

Figure 1 Chemiluminescence with Vizilite™ shows suspicious lesion on the floor of the mouth as white (arrow); normal epithelium is dark (http://www.vizilite.com/UserFiles/File/ViziliteCaseStudies_02.pdf)

disease process, the altered cellular structure (e.g., hyperkeratosis, hyperchromatin and increased cellular/nuclear pleomorphism) and/or metabolism (e.g. concentration of flavin adenine dinucleotide and nicotinamide adenine dinucleotide) affect tissue interaction with light. Spectroscopy or autofluorescence imaging can provide information about these altered light interaction properties.

In the last decade, several forms of autofluorescence technology have been developed for inspection of the oral mucosa. LED Medical Diagnostics Inc. in partnership with the British Columbia Cancer Agency has marketed the VELscope™ system (LED Dental Inc., White Rock, BC, Canada) (De Veld *et al*, 2005; Lingen *et al*, 2008; Patton *et al*, 2008). When viewed through the instrument eye piece, normal oral mucosa emits a pale green autofluorescence upon stimulation with intense blue excitation at 400–460 nm wavelength, while dysplastic lesions exhibit decreased autofluorescence and appear darker with respect to the surrounding healthy tissues (Figure 2). Several studies have investigated the effectiveness of the VELscope™ system as an adjunct to visual examination, and determined an improvement in the ability to distinguish between oral lesions and healthy mucosa, and between different lesion types (De Veld *et al*, 2005). Overall, the technique seems to show high sensitivity, but low specificity (De Veld *et al*, 2005). Using histology as the comparative gold standard, VELscope™ demonstrated high sensitivity and specificity in identifying areas of dysplasia and malignancy that extended beyond the clinically evident tumors (Onizawa *et al*, 1996; Schantz *et al*, 1998; De Veld *et al*, 2005; Lingen *et al*, 2008; Patton *et al*, 2008). A direct clinical application consists of assessing pathology margins in patients with potentially malignant oral lesions, therefore assisting in guiding surgical management (Poh *et al*, 2007; Rosin *et al*, 2007). However, reported evaluations of the VELscope™ system are from case series and case reports rather than clinical trials, and no published studies have assessed the VELscope™ system as a diagnostic adjunct in screening patient populations [including patients with or without a history of dysplasia/oral squamous cell carcinoma (OSCC)].

In another study using *quantitative* fluorescence imaging in 56 patients with oral lesions and 11 normal volunteers, healthy tissue could be discriminated from dysplasia and invasive cancer with 95.9% sensitivity and 96.2% specificity in the training set, and with 100% sensitivity and 91.4% specificity in the validation set. Lesion probability maps qualitatively agreed with both clinical assessment and histology (Roblyer *et al*, 2009). Further clinical studies are needed in diverse populations to evaluate fully the clinical usefulness of this promising technology.

Other devices using a range of spectroscopic techniques are under development, often combined with other technologies. These include the FastEEM4™ System, the Indentafi™ (Trimira® Remicalm, Houston, TX, USA) and the PS2-oral™ (Farrell *et al*, 1992; Bigio and Mourant, 1997; Wagnieres *et al*, 1998; Ramanujam, 2000; Choo-Smith *et al*, 2002; Culha *et al*, 2003; De Veld *et al*, 2005; Lane *et al*, 2006; McGee *et al*, 2008;

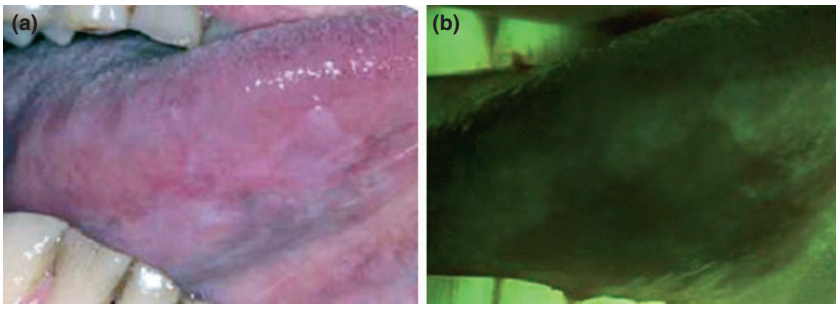

Figure 2 Direct visualization of leukoplakia under white light (LHS) and VELscope™ examination (RHS). (**a**) Visible leukoplakia. (**b**) Dark area visible under fluorescence visualization. Biopsy confirmed severe dysplasia in this area (http://www.vizilite.com/UserFiles/File/ViziliteCaseStudies_02.pdf)

Schwarz *et al*, 2009). Clinical studies are still at a relatively early stage, and preliminary results are encouraging. The Remicalm's Identafi™ technology combines anatomical imaging with fluorescence, fiber optics and confocal microscopy to map and delineate precisely the lesion in the area being screened. In a screening of 124 subjects, a sensitivity of 82% and a specificity of 87% were determined for differentiating between neoplastic and non-neoplastic sites in the oral cavity. Results appeared to vary between sampling depths, and keratinized *vs* non-keratinized tissues (Schwarz *et al*, 2009).

Major challenges to diagnostic spectroscopy include the often low signal-to-noise ratio, difficulty in identifying the precise source of signals, data quantification, and difficulty in establishing definitive diagnostic milestones and endpoints, especially given the wide range of tissue types within the oral cavity. The depth of tissue penetration is an inherent limitation of the technology. Additional concerns relate to the potential mutagenicity induced by UV light in the clinical setting.

In vivo microscopy: confocal and multiphoton imaging This imaging modality resembles histological tissue evaluation in concept. However, three- dimensional subcellular resolution is achieved noninvasively and without the need for staining. In epithelial structures, resolutions of 1 μm have been achieved with a 200–400 μm field of view (White *et al*, 1999; Clark *et al*, 2003; Wilder-Smith *et al*, 2004). While this technology can provide detailed images of tissue architecture and cellular morphology, cost, the relatively minuscule field of view and limited penetration depth reduce its clinical usefulness.

Photosensitizers When topical or systemic photosensitizers are administered, their ability to accumulate in cancer cells and to fluoresce under specific wavelengths can be used to identify and delineate areas of microscopic changes (Kennedy and Pottier, 1992; Cassas *et al*, 2002). This approach permits 3D mapping of the epithelial surface and sub-epithelial boundary, screening of large surface areas and offers the option of subsequent photodestruction of the photosensitized lesion. Many photosensitizing agents have been studied; however, FDA approval for photosensitizing drugs remains limited. Some promising agents for photodetection include aminolevulinic acid (Levulan®; DUSA Pharmaceuticals, Inc., Wilmington, MA, USA), hexyl aminolevulinate (Hexvix®; Photocure ASA, Oslo, Norway), methyl aminolevulinate (MetvixR; Photocure ASA), tetra (meta-hydroxyphenyl) chlorin (mTHPC®; Biolitec Pharma Ltd., Dublin, Ireland), as well as porfimer sodium (Photofrin®; Axcan Pharma, Birmingham, AL, USA) (Leunig *et al*, 1996, 2000, 2001; Ebihara *et al*, 2003; Chang and Wilder-Smith, 2005). In a blinded clinical study of 20 patients with oral neoplasms (Figure 3), 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 (Chang and Wilder-Smith, 2005).

Depending on the photosensitizer and its mode of application (systemic *vs* topical), limitations include systemic photosensitization over prolonged periods of time, penetration-related issues, the need for specialized fluorescence detection and mapping equipment, and lack of specificity when inflammation or scar tissues are present.

A recent study using epidermal targeted fluorescent agents by topical applications to oral mucosal lesions combined with in-vivo imaging showed encouraging results with regard to lesion detection, margin delineation and as an adjunct guiding tool for biopsy (Nitin *et al*, 2009).

Optical coherence tomography Optical coherence tomography (OCT) was first introduced as an imaging technique in biological systems in 1991 (Huang *et al*, 1991). The non-invasive nature of this imaging modality coupled with (i) a penetration depth of 2–3 mm, (ii) high resolution (5–15μm), real-time image viewing, and (iii) capability for cross-sectional as well as 3D tomographic images, provide excellent prerequisites for *in vivo* oral screening and diagnosis.

Optical coherence tomography has most often been compared with ultrasound imaging. Both technologies employ back-scattered signals reflected from different layers within the tissue to reconstruct structural images, with the latter measuring sound rather than light. The resulting OCT image is a two-dimensional representation of the optical reflection within a tissue sample. Cross-sectional images of tissues are constructed in real time, at near histologic resolution (approximately

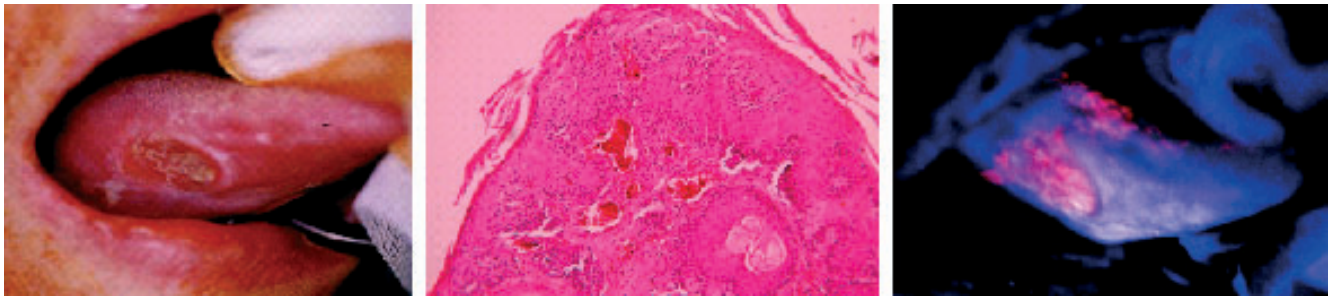

Figure 3 Photograph, histology and *in vivo* fluorescence images of a tongue with multiple squamous cell carcinoma lesions: Photofrin®-induced red fluorescence is clearly apparent in multiple lesions of the tongue. From Chang and Wilder-Smith (2005). Reprinted with permission

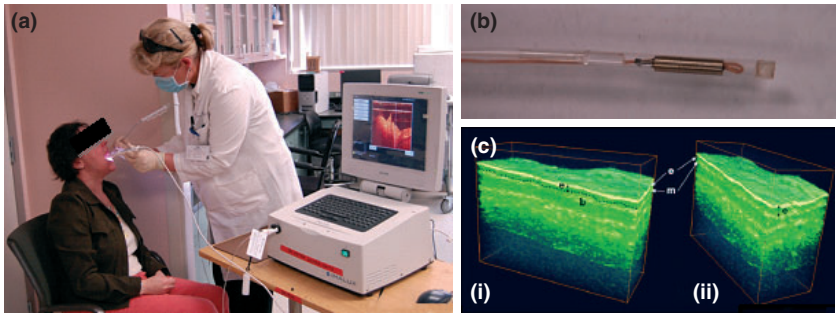

Figure 4 (**a**) Intra-oral imaging using the Imalux^{TM} OCT system (Imalux Corp., Cleveland, OH, USA). (**b**) Photograph of intra-oral fiberoptic probe (Reproduced with permission: Jun Zhang and Zhongping Chen for 3D imaging). (**c**) In the 3D reconstructed OCT images of healthy hamster cheek pouch mucosa, the surface squamous keratinized epithelium, and underlying submucosa and muscle layer are clearly visible (A: Anterior view; B: Lateral view; e: epithelium; b: basement membrane; m: mucosa). From: Jung WG, Zhang J, Chung JR, Wilder-Smith P, Brenner M, Nelson JS, Chen Z (2005). Advances in oral cancer detection using optical coherence tomography. *J Biophotonics STQE* **11**: 811–817. Reprinted with permission

5–15 μm with current technology). These images can be stacked to generate 3D reconstruction of the target tissue. This permits *in vivo* non-invasive imaging of epithelial and subepithelial structures, including: (i) depth and thickness, (ii) histopathological appearance, and (iii) peripheral margins of the lesions.

Several OCT systems have received FDA approval for clinical use, and OCT is deemed by many as an essential imaging modality in ophthalmology. *In vivo* image acquisition is facilitated through the use of a flexible fiber optic OCT probe (Figure 4). The probe is simply placed on the surface of the tissue to generate real-time, immediate surface and sub-surface images of tissue microanatomy and cellular structure, while avoiding the discomfort, delay and expense of biopsies.

Several studies have sought to investigate the diagnostic utility of *in vivo* OCT to detect and diagnose oral pre-malignancy and malignancy (Tsai *et al*, 2008a,b; Wilder-Smith *et al*, 2009a,b). In a blinded study involving 50 patients with suspicious lesions including oral leukoplakia or erythroplakia, the effectiveness of OCT was evaluated for detecting oral dysplasia and malignancy (Wilder-Smith *et al*, 2009a,b). OCT images of dysplastic lesions revealed visible epithelial thickening, loss of epithelial stratification, and epithelial down-growth (Figure 5). Areas of OSCC of the buccal mucosa were identified in the OCT images by the absence or disruption of the basement membrane, an epithelial layer that was highly variable in thickness, with areas of erosion and extensive epithelial down-growth and invasion into the sub-epithelial layers (Figure 6). Statistical analysis of the data gathered in this study substantiated the facility of *in vivo* OCT to detect and diagnose oral pre-malignancy and malignancy in the oral cavity, with excellent diagnostic accuracy. For detecting carcinoma *in situ* or squamous cell carcinoma (SCC) *vs* non-cancer, sensitivity was 0.931 and specificity was 0.931; for detecting SCC *vs* all other pathologies, sensitivity was 0.931 and specificity was 0.973.

In another study of 97 patients using OCT imaging to detect neoplasia in the oral cavity (Tsai *et al*, 2009), the results revealed that the main diagnostic criterion for high-grade dysplasia/carcinoma *in situ* was the lack of a layered structural pattern. Diagnosis based on this criterion for dysplastic/malignant *vs* benign/reactive conditions achieved a sensitivity of 83% and specificity of 98% with an inter-observer agreement value of 0.76. This study concluded that OCT, with high sensitivity and specificity combined with good inter-observer agreement, is a promising imaging modality for non-

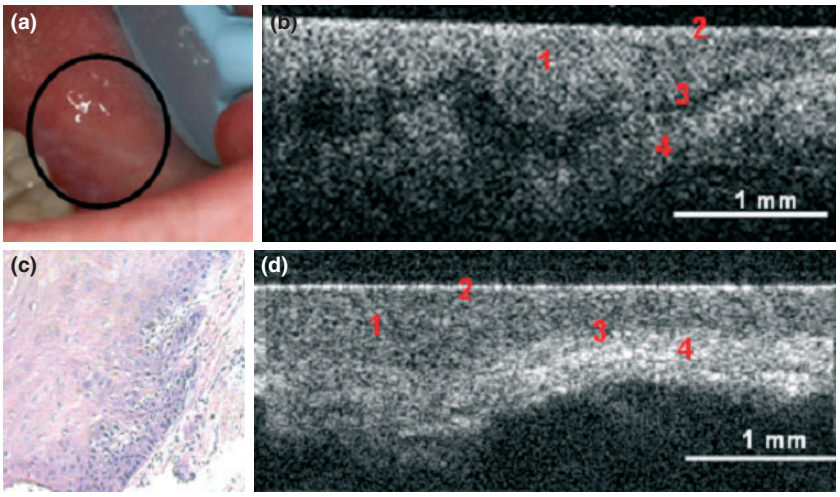

Figure 5 Dysplastic and normal buccal mucosa: (**a**) Photograph, (**b**) *in vivo* optical coherence tomography (OCT) image, and (**c**) H&E (10×) of dysplastic buccal mucosa. (**d**) *In vivo* OCT image of normal buccal mucosa. (1: stratified squamous epithelium; 2: keratinized epithelial surface layer; 3: basement membrane; 4: submucosa). From Wilder-Smith *et al* (2009b). Reprinted with permission of Wiley-Liss, Inc. a subsidiary of John Wiley & Sons, Inc

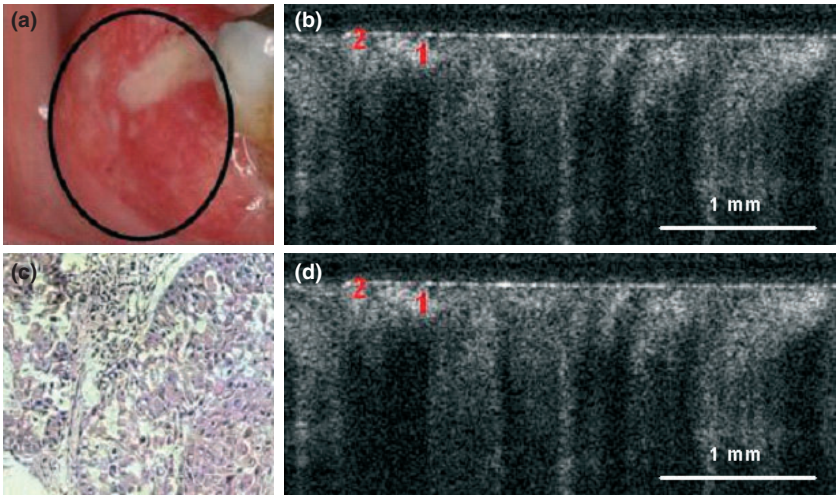

Figure 6 Squamous cell carcinoma of the buccal mucosa: (**a**) photograph, (**b**) *in vivo* optical coherence tomography (OCT) image and (**c**) H&E (10×) of buccal mucosa with squamous cell carcinoma. (**d**) *In vivo* OCT image of normal buccal mucosa (1: stratified squamous epithelium; 2: keratinized epithelial surface layer; 3: basement membrane; 4: submucosa). From Wilder-Smith *et al* (2009b). Reprinted with permission of Wiley-Liss, Inc. a subsidiary of John Wiley & Sons, Inc

invasive evaluation of tissue sites suspicious for high-grade dysplasia or cancer.

Other studies have utilized direct analysis of OCT scan profiles, rather than image-based criteria, as a means of delineating the site and margins of oral cancer lesions (Tsai *et al*, 2008a,b). Using numerical parameters from A-scan profiles as diagnostic criteria, the decay constant in the exponential fitting of the OCT signal intensity along the tissue depth decreased as the A-scan point moved laterally across the margin of a lesion. Additionally, the standard deviation of the OCT signal intensity fluctuation increased significantly across the transition region between the normal and abnormal portions. The authors concluded that such parameters may well be useful for establishing an algorithm for detecting and mapping the margins of oral cancer lesions. Such a capability has huge clinical significance, because of the need to better define excisional margins during surgical removal of oral pre-malignant and malignant lesions.

Cancer therapy-induced mucositis

Oropharyngeal mucositis (OM) occurs in 30–75% of chemotherapy patients, 75% of patients receiving hematopoietic cell transplant, and in essentially all patients receiving head and neck radiation therapy in doses over 5000 cGy. Currently, prediction of onset and severity of mucositis is not possible, thereby hampering efforts at targeted intervention and optimizing treatment outcomes. The ability to detect OM at the early stage, monitor and characterize disease progress would greatly enhance our understanding of the pathogenesis of mucositis, leading to improved preventive and treatment strategies.

Two animal studies of OM were carried out using several optical imaging technologies to detect mucositic changes several days before full clinical manifestation, or in cases whereas the mucositic damage remained subclinical (Muanza *et al*, 2005; Wilder-Smith *et al*, 2007). OCT combined with optical Doppler tomography detected *in vivo* microstructural changes as well as considerable alterations in axial blood flow velocity. An imaging-based scoring system was developed for the semi-quantification of early, intermediate and late mucositic changes. Using this scoring system, imaging data consistently gave higher scores compared with clinical scores at the early stage of the disease, suggesting that the imaging-based diagnostic tool was more sensitive to detect early mucositic changes than the current clinical approach. Once mucositis was established, imaging and clinical scores converged.

In a preliminary study, oral mucositis was assessed clinically and imaged using non-invasive OCT in five patients receiving neoadjuvant chemotherapy for primary breast cancer. Imaging was scored in a blinded fashion using a novel imaging-based scoring system. Using OCT, the following changes were observed prior to clinical manifestation of mucositis (Figure 7): (i) altered epithelial thickness and subepithelial tissue integrity (beginning on day 2), (ii) loss of surface keratinized layer continuity (beginning on day 4), and (iii) loss of epithelial integrity (beginning on day 4). In agreement with findings from the animal model, imaging data from this pilot clinical study gave higher scores earlier in treatment when compared with clinical scores using the Oral Mucositis Assessment Scale (OMAS); however, once mucositis was established, imaging and clinical scores converged (Kawakami-Wong *et al*, 2007).

Other oral lesions with potential benefits from OCT

As OCT can delineate subtle changes in epithelial thickening, stratification, sub-epithelial continuity and integrity, this imaging tool has potential broad applications in other mucosal lesions associated with inflammatory or immune diseases, including lichen planus, pemphigus, pemphigoid, lupus erythematosus, graft-*vs*-host disease, or anomalies such as vascular lesions.

Optical coherence tomography images of oral vascular anomalies have recently been reported in two cases, one with a capillary–venous malformation of the lower lip and the other with a reddish mass of the buccal mucosa (Ozawa *et al*, 2009). In these cases, OCT images correlated well with histological structures, showing nicely demarcated capillary vessel lumina and endothelial lining. Knowledge of the size, area, and border of the

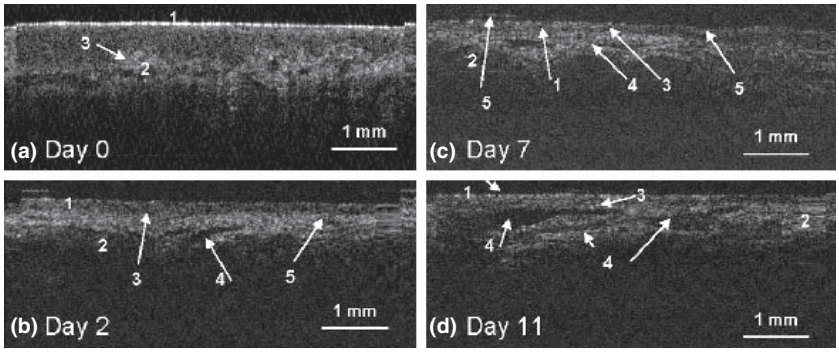

Figure 7 *In vivo* optical coherence tomography (OCT) images throughout the development of oral mucositis: OCT images of ventral surface of tongue before (**a**), after 2 days (**b**), after 7 days (**c**) and after 11 days (**d**) of chemotherapy. In (**a**), smooth stratified squamous epithelium (1) is visible, separated from the submucosa (2) by the basement membrane (3). Cumulative diagnostic imaging score is 0. In (**b**), epithelium is thinner by 50%, surface is still intact, although directly below the surface some breakdown is apparent (5). Subepithelial tissues just below the basement membrane show some disruption. At this point, the patient was totally asymptomatic. Cumulative diagnostic imaging score is 2. Further epithelial atrophy is seen after 7 and 11 days (**c, d**), with infiltrate around the basement membrane and disruption of the adjacent epithelial and subepithelial tissues (4), and breakdown of the epithelial surface (5). Cumulative diagnostic imaging score for (**c**) is 3 and for (**d**) is 5. From Kawakami-Wong *et al* (2007). Reprinted with permission

vascular lesions can be useful for the diagnosis and choice of surgical treatment, particularly for hemangiomas and vascular anomalies in the orofacial region. The high resolution in real-time, the lack of invasion, and its ease of handling provide a safe means of imaging oral structure at a level that is otherwise only obtainable by histological examination of a biopsy specimen. Moreover, the emerging capability for combining *in vivo* OCT with *in vivo* Doppler techniques for imaging and measuring perfusion is very attractive.

In the head and neck region, most interest in the use of OCT has been focused on the early detection and monitoring of dysplastic and cancerous conditions to facilitate less aggressive treatment and a better prognosis. In a recent study, comparison between OCT with histological findings of 'suspicious oral lesions'' were carried out to determine whether this technique would aid clinical examination and monitoring of these lesions which would not normally warrant a biopsy (Jerjes *et al*, 2009). Based on four variables (changes in keratin, epithelial, sub-epithelial layers, and identification of the basement membrane) and overall architectural changes, 34 oral lesions were evaluated using OCT and compared with histological results. The basement membrane was recognized in 15 oral lesions. OCT could identify diseased areas but could not provide a diagnosis or differentiate between lesions. One of the lesions was a fluid filled vesicle of the lower lip, which was nicely delineated by OCT with defined keratin, epithelial layer, and lamina propria. Another case was a vesiculobullous lesion of the buccal mucosa with unclear basement membrane outline which correlated with the histopathological features of the disease. When imaging a plaque of the cheek mucosa, there was discontinuity in the keratin layer with epithelial ulceration and areas of keratosis and thickening suggestive of submucous fibrosis. This pilot study confirms the feasibility of using OCT as an adjunct imaging tool for diagnosis and longitudinal monitoring of several benign as well as suspicious oral lesions without the need for multiple biopsies. This non-invasive tool is less morbid to patients and allows preventive screening of high-risk populations.

Dental pathologies and other applications

Light scattering, reflection, absorption, and laser-induced fluorescence can provide much information regarding hard tissue structure and pathology. The techniques described below including (i) OCT, (ii) polarization-sensitive (PS) OCT, (iii) laser fluorescence (KaVo DIAGNOdent™), (iv) quantitative laser fluorescence, (QLF™), (v) DIFOTI™ fiber optic transillumination take advantage of this concept, achieving varying degrees of specificity and sensitivity for detecting demineralization and decay of the dental matrices, the anatomical structure of the tooth organ, as well as the attached microbial biofilms and calculus.

Dental caries

Optical coherence tomography As described above, OCT measures the intensity of back-scattered light to create images. Light does not travel at a constant velocity when it passes through different structures, traveling faster in material with a low refractive index and slower in media with a high refractive index. Additionally, when the light hits a sharp change in refraction, the wave is reflected either externally or internally. The amount of reflection depends on the amount of change in refraction, the angle the light is traveling at and the polarization of the light. If the change of refraction between the media is gradual, the reflection will be minimal (Brezinski, 2006; Colston *et al*, 1998a,b; Feldchtein *et al*, 1998; Otis *et al* (2000). The changes between the hard tissues such as enamel and dentin and between healthy and demineralized or carious states can then be interpreted to create 2D and 3D images of the hard tissues. As such, various optical properties are under investigation as potential quantifiers of the mineralization changes to detect dental caries (Figure 8) (Li *et al*, 2009).

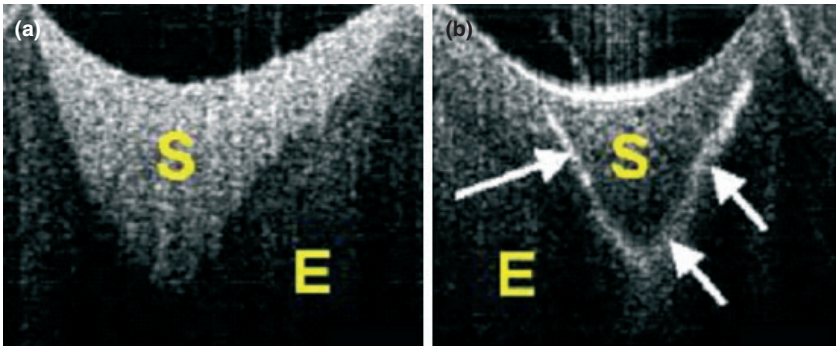

Figure 8 *Ex vivo* optical coherence tomography images of tooth with sealant. (**a**) Sealant (S) over healthy enamel (E). (**b**) Sealant (S) over decay (arrow)

To date, few reports have been published on the use of OCT for *in vivo* dental diagnosis. In the relatively early days of OCT, two groups of researchers investigated the feasibility of using OCT *in vivo* to image sound and demineralized tissues, and even monitored restorative procedures (Colston *et al*, 1998a,b). A recent publication described *in vivo* OCT to determine the effectiveness of a proton pump inhibitor to treat gastro-esophageal reflux (GERD) by monitoring dental erosion with OCT (Wilder-Smith *et al*, 2009a,b). The study was significant in that researchers were able to identify an association between the medication and a reduction in enamel erosion.

Polarization-sensitive OCT As both enamel and dentin have strong polarizing effects, changes in polarization provide more structural information than conventional OCT (Brezinski, 2006). Light is delivered in one polarization, and the reflection is read in both polarizations. Although we were unable to find clinical studies using PS-OCT, extensive research has been conducted by Fried and others demonstrating that this technology has the potential to monitor demineralization/remineralization, and quantify demineralized tooth structure even below dental sealant (Manesh *et al*, 2009; Chen *et al*, 2005; Jones *et al*, 2006 Jones and Fried (2006), Ngaotheppitak *et al*, 2005; Chong *et al*, 2007; Jones *et al*, 2004). Unfortunately PS-OCT technology has not been as effective in identifying root caries (Lee *et al*, 2009).

Laser fluorescence [KaVo DIAGNOdent™ (KaVo Dental, Charlotte, NC, USA)] Back-scattered light from laser-induced fluorescence has been reported as a tool to detect and quantify caries activity (Zandoná and Zero, 2006). A red laser light (655 nm wavelength) is absorbed by organic and inorganic matter in the tooth and then re-emitted from the organic material as near-infrared fluorescent light. The device provides a numerical read-out as well as an audible signal when decay is detected. The results of studies investigating diagnostic usefulness of DIAGNOdent™ (KaVo, Biberach, Germany) vary considerably (Chong *et al*, 2003; Kühnisch *et al*, 2008). The lack of diagnostic consistency may reflect: (i) the need for clinicians to learn how to use the correct position for the unit; (ii) staining and/or calculus affecting the readings; and (iii) difficulty in determining the numerical value at which surgical intervention is indicated (Shi *et al*, 2000). However, the literature seems to be consistent in describing DIAGNOdent™ as a better tool for detecting dentinal caries than enamel caries. Additional benefits of the DIAGNOdent™ may be its ability to identify completed removal of infected tooth structure during excavation (Lussi *et al*, 2004). Although DIAGNOdent™'s high rate of false-positive results may be a limitation in some clinical practices, in a high-risk population with limited access to dental care this tool may be quite predictive in caries screening.

Quantitative light fluorescence (QLF™; Inspektor Dental Care, Amsterdam, Netherlands) QLF™ uses fluorescence induced by multi-wavelength excitation at 290–450 nm wavelength to measure mineral loss in enamel and dentin (Hall and Girkin, 2004). Unlike the Diagnodent system, this device provides color-coded images of the target tissues. Sound tooth structure fluoresces and carious tooth structure appears dark (Figure 9). As the caries scatters the light, mapping the carious lesion can be difficult. Interestingly, the predictive nature of this technology depends on the population (Hall and Girkin, 2004). In a high-risk population, QLF™ is highly predictive (0.90–0.98) of future caries (Zandoná and Zero, 2006). In a low-risk population, it is much less predictive. Stains, plaque, and fluorosis can affect QLF™ accuracy (Zandoná and Zero, 2006). High intensity UV light can generate free radicals, potentially resulting in toxicity to live tissues.

Fiber optic trans illumination (FOTI) This approach uses changes in the scattering and absorption of photons by structural characteristics to detect caries in real time. Advantages of this technology include safety, as UV light is not used. In Digital Image Fiber Optic Trans Illumination (DIFOTI™; KaVo Dental, Lake Zurich, IL, USA) the light that passes through the tooth is interpreted by a digital device on the other side of the tooth. DIFOTI™ seems to perform well for early surface lesions; however, it seems to have low specificity which can result in over-treatment, and is also unable to determine lesion depth, which limits potential sites of use (Schneiderman *et al*, 1997; Young and Featherstone, 2005; Bin-Shuwaish *et al*, 2008). Recently, Wu and Fried (2009) used near infra-red (NIR) transillumination to image dental caries. This technology takes advantage

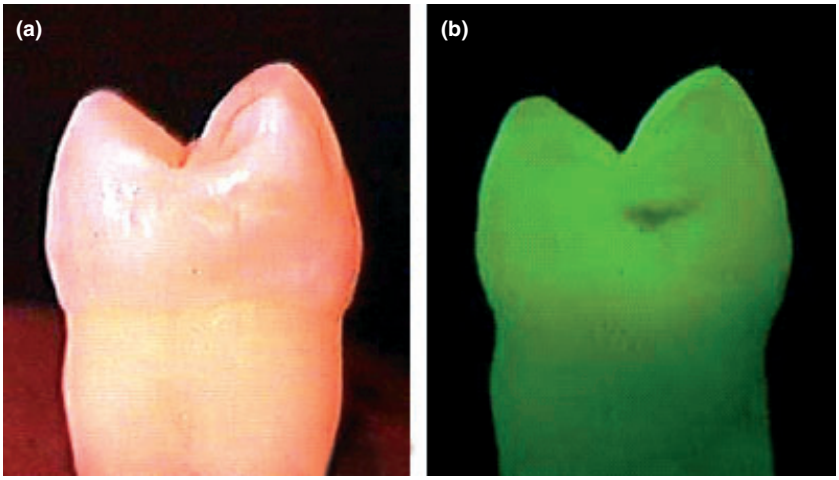

Figure 9 Images of tooth with early lesion viewed using white light and QLF™. (**a**) Tooth image without QLF™. Visually, the enamel lesion is difficult to detect. (**b**) Tooth image with QLF™. The enamel lesion becomes apparent in the QLF™ image

of the transparency of sound enamel at 1310 nm, which decreases considerably in unhealthy tooth structure. Demineralized areas on the enamel surface appear lighter, while deeper lesions appear darker. However, low contrast compared with the high reflectance signal, and decreasing effectiveness with increasing tooth thickness are important clinical challenges. Although we have been unable to identify clinical studies using NIR transillumination, the concept holds great promise, for example, allowing clinicians to monitor remineralization of enamel.

Other dental applications

Periodontics Fluorescence using the Perio Probe for DIAGNOdent™: Because calculus fluoresces differently than healthy tissue, the use of laser fluorescence has been proposed as an aid to detect residual calculus following root planing and scaling. The KAVO *Perio Probe for DIAGNOdent*™ may aid in clinical detection of subgingival calculus deposits far better than conventional methods (Krause *et al*, 2003, 2005; Kasaj *et al*, 2008). Audible sounds and measurable values as signals for presence of calculus during screening may increase patients' awareness of their calculus levels, leading to increased patient compliance with the recommended treatment.

Optical coherence tomography: Several *in vitro* studies have demonstrated the potential use of OCT as an adjunct tool for diagnosis of periodontal disease. Studies in a porcine model showed high-resolution images of periodontal tissues, the enamel–cementum, and the gingiva–tooth interfaces (Colston *et al*, 1998a,b). Although results of early *in vivo* studies were promising, consistent imaging of the periodontal tissues remains challenging because of the limited penetration depth and scan sizes of OCT (Colston *et al*, 1998a,b). In another study by Baek *et al* (2009), the successful use of OCT for monitoring periodontal ligament changes during orthodontic tooth movements was reported in rats (Figure 10).

Endodontics Fluorescence using the Perio Probe for DIAGNOdent™: Real-time assessment of the microbial status of the root canal system would be useful in clinical endodontic practice for determining endpoints of biomechanical treatment. In an *ex vivo* study using extracted teeth, the DIAGNOdent™, in combination with a prototype sapphire tip designed for periodontal assessment, were used to evaluate the pulp chamber and coronal third of the root canal system. The fluorescence properties of bacterial colonies, biofilms in root canals, pulpal soft tissues, and sound dentin were evaluated in 50 extracted teeth with known endodontic pathology. Sound dentin and healthy pulpal soft tissues gave an average fluorescence reading of 5 (on a scale of 100), whereas biofilms of *Enterococcus faecalis* and *Streptococcus mutans* colonizing the root canals showed a progressive increase in fluorescence signals over time.

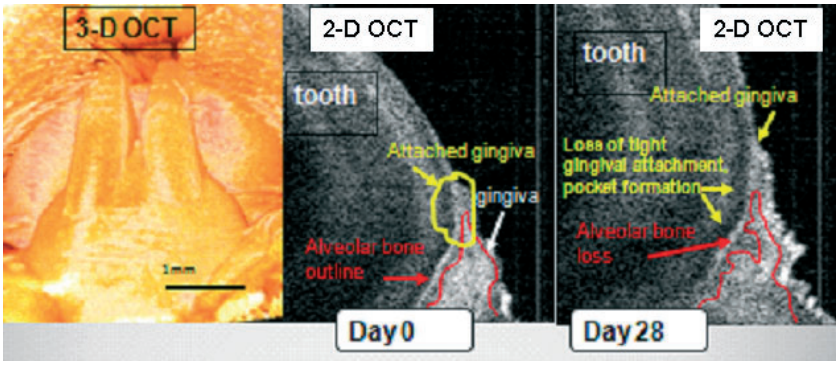

Figure 10 In a diabetic strain of mice, the progression of gingivitis/periodontitis was effectively imaged and monitored using *in vivo* optical coherence tomography

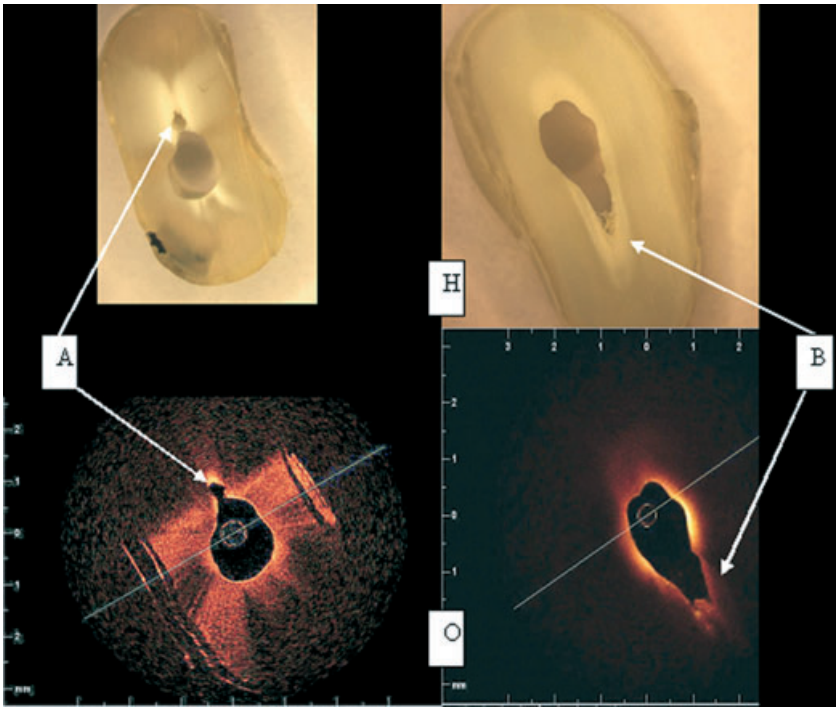

Figure 11 Oval canals and uncleaned fins at 7 mm from the apex revealed by histology (H) and optical coherence tomography (O). Oval canals (A) and canal fins (B)

Fluorescence readings reduced to the 'healthy' threshold range when root canals were endodontically treated, and the experimentally created bacterial biofilms were removed completely. High fluorescence readings were recorded in the root canals and pulp chambers of extracted teeth with radiographic evidence of periapical pathology and scanning electron microscopy evidence of bacterial infection (Sainsbury *et al*, 2009).

Magnification devices: Several magnification devices have been introduced in endodontics, including loupes, surgical microscopes (Pecora and Andreana, 1993; Khayat, 1998; Rubinstein and Kim, 1999; Castellucci, 2003) and, more recently, endoscopes (Bahcall *et al*, 1999; Bahcall and Barss, 2000, 2003; von Arx *et al*, 2002). Besides increasing the accuracy of the endodontic procedure, these devices may improve diagnostic capability, as a result of a better visualization of the treatment field. As an example, they permit identification of isthmuses, accessory canals or microfractures of the root, that are difficult to recognize and treat in the absence of proper magnification (Coelho de Carvalho and Zuolo, 2000; Schwartze *et al*, 2002; von Arx *et al*, 2003; Slaton *et al*, 2003; Rampado *et al*, 2004; von Arx, 2005). Several *in vitro* studies have confirmed that magnification devices such as the microscope or the endoscope provide a more reliable means of identifying dental microstructures not visible with the naked eye (Coelho de Carvalho and Zuolo, 2000; Görduysus *et al*, 2001; Baldassari-Cruz *et al*, 2002; Schwartze *et al*, 2002; von Arx *et al*, 2003; Slaton *et al*, 2003; Zaugg *et al*, 2004). However, the clinical benefits in terms of improved outcomes of using aids such as a microscope, an endoscope, or surgical loupes *vs* the naked eye remain to be proven (Del Fabbro *et al*, 2009).

Optical coherence tomography: In a study on extracted teeth, the diagnostic accuracy of high-resolution OCT using a 0.5 mm diameter intra-canal probe for mapping oval canals, uncleaned fins, risk zones, and root perforations approached that provided by histology (Shemesh *et al*, 2007). The probe easily fitted into a prepared root canal and its flexibility allowed penetration and advancement through curvatures. The optical probe rotated within a probe sheath so that adjacent lines in each rotation could be stacked to generate a frame showing a cross-section of the tissue architecture in the wall. The scan was quick, about 15 s for a 15 mm-long root. The authors concluded that fiberoptic OCT probing holds promise for full *in vivo* endodontic imaging (Figure 11).

Another *ex vivo* study assessed apical microleakage following endodontic treatment using OCT (Todea *et al*, 2009). OCT imaging was found to be effective in identifying the apical seal. However, in the real clinical situation, OCT use for periapical diagnostics is limited by its short penetration depth into the bone in which the tooth is embedded.

Conclusion

Emergent optical technologies show promise for a wide range of oral diagnostic applications with capabilities for high-resolution, cross-sectional tomographic imaging of microstructure in several biological systems. OCT can achieve image resolution one to two orders of magnitude finer than standard ultrasound. As such, OCT functions more effectively as a unique 'optical biopsy' to delineate the cross-sectional images of tissue structure at the micro scale. This promising biomedical

optical imaging technology provides images of tissue *in situ* and in real time, without the need for surgical biopsy and multiple specimen processing. OCT imaging allows detection and diagnosis of early stages of disease in teeth, periodontal tissue, and mucosa, and facilitates large scale screening for high-risk populations. Because of the rapid pace of innovation in this field, the cost and ease of use of such modalities is improving rapidly, so that many such devices are becoming available to dental clinicians. We envisage many benefits to patients and clinicians from the use of these devices.

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