Optical imaging in the oral cavity

Innovative and emergent imaging techniques

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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 becomes 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: (a) non-invasiveness; (b) absence of ionising radiation; (c) patient friendly; (d) real-time information; (e) repeatability; and (f) high-resolution surface and subsurface images. In this article, the principles behind optical diagnostic approaches, their feasibility and applicability to imaging soft and hard tissue, and their potential usefulness as a tool in the diagnosis of oral mucosal lesions, dental pathologies, and for other dental applications will be reviewed.

_Introduction

Light-based imaging of tissue detects minimal changes, such as: (a) cell microanatomy (e.g. nuclear/cytoplasmic ratio); (b) redox status; (c) expression of specific biomarkers; (d) tissue architecture and composition; (e) chemical changes (e.g. mineralisation); and (f) 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.

_Oral cancer

A. Chemiluminescence: ViziLite

This imaging device has been used in the oral cavity since 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; Kerr *et al.* 2006; Epstein *et al.* 2006; Epstein *et al.* 2008). 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. Hyper-keratinised or dysplastic lesions appear distinctly white when viewed under a diffuse low-energy wavelength light. In contrast, normal epithelium will absorb light and appear dark (Lingen *et al.* 2008). Since the majority of studies investigating chemiluminescence reported subjective perceptions of intra-oral lesions in terms of brightness, sharpness and texture versus 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) 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, AdDent) has been introduced as an adjunct tool for oral lesion identification (McIntosh & Farah 2009).

B. 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 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 (Lingen *et al.* 2008; Patton *et al.* 2008; De Veld *et al.* 2005). When viewed through the instrument eyepiece, normal oral mucosa emits a pale green autofluorescence upon stimulation with intense blue excitation at 400 to 460 nm wavelength, whilst dysplastic lesions exhibit decreased autofluorescence and appear darker with respect to the surrounding healthy tissue. 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 appears 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 tumours (Lingen *et al.* 2008; Patton *et al.* 2008; De Veld *et al.* 2005; Onizawa *et al.* 1996; Schantz *et al.* 1998). A direct clinical application entails assessing pathology margins in patients with potentially malignant oral lesions, thereby 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).

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 a sensitivity of 95.9 % and specificity of 96.2 % in the training set, and with a sensitivity of 100 % and specificity of 91.4 % 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 Identafi (Remicalm) and the PS2-oral (Schwarz *et al.* 2009; McGee *et al.* 2008; Lane *et al.* 2006; De Veld *et al.* 2005; Wagnieres *et al.* 1998; Ramanujam *et al.* 2000; Culha *et al.* 2003; Choo-Smith *et al.* 2002; Bigio *et al.* 1997; Farrell *et al.* 1992). Clinical studies are still at a relatively early stage, but preliminary results are encouraging. The Identafi technology combines anatomical imaging with fluorescence, fibre 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 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 keratinised versus non-keratinised tissue (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.

C. Photosensitisers

When topical or systemic photosensitisers 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 *et al.* 1992; Cassas *et al.* 2002). This approach permits 3-D mapping of the epithelial surface and subepithelial boundary, screening of large surface areas and offers the option of subsequent photodestruction of the photosensitised lesion. Some promising agents for photodetection include aminolevulinic acid (Levulan), hexyl aminolevulinate (Hexvix), methyl aminolevulinate (Metvix), tetra(meta-hydroxyphenyl)chlorin, as well as porfimer sodium (Photofrin; Ebihara *et al.* 2003; Leunig *et al.* 1996, 2000, 2001; Chang & Wilder-Smith, 2005). In a blinded clinical study of 20 patients with oral neoplasms, 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 & Wilder-Smith, 2005). A recent study using epidermal growth factor-targeted fluorescent agents by topical application to oral mucosal lesions, combined with *in vivo* imaging, demonstrated encouraging results with regard to lesion detection, margin delineation and as an adjunct guiding tool for biopsy (Nitin *et al.* 2009). Depending on the photosensitiser and its mode of application (systemic versus topical), limitations include systemic photosensitisation over prolonged periods, penetration-related issues, the need for specialised fluorescence detection and mapping equipment, and lack of specificity when inflammation or scar tissue is present.

D. 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 a penetration depth of 2 to 3 mm, high resolution (5–15 µm), real-time image viewing and capability for cross-sectional, as well as 3-D tomographic images, provides excellent prerequisites for *in vivo* oral screening and diagnosis. OCT has frequently been compared to 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 2-D representation of the optical reflection within a tissue sample. Cross-sectional images of tissue are constructed in real time, at near histological resolution (approximately 5–15 µm with current technology). These images can be stacked to generate a 3-D reconstruction of the target tissue. This permits *in vivo* non-invasive imaging of epithelial and subepithelial structures, including depth and thickness, histopathological appearance and peripheral margins of the lesions.

Several OCT systems have received US 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 fibre-optic OCT probe. The probe is simply placed on the surface of the tissue to generate real-time, immediate surface and subsurface images of tissue microanatomy and cellular structure, whilst 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.* 2008; Wilder-Smith *et al.* 2009). In a blinded study involving 50 patients with suspicious lesions, including oral leukoplakia and erythroplakia, the effectiveness of OCT for detecting oral dysplasia and malignancy was evaluated (Wilder-Smith *et al.* 2009). OCT images of dysplastic lesions revealed visible epithelial thickening, loss of epithelial stratification, and epithelial downgrowth. Areas of oral squamous cell carcinoma 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 downgrowth and invasion into the subepithelial layers. Statistical analysis of the data gathered in this study substantiated the ability of *in vivo* OCT to detect and diagnose pre-malignancy and malignancy in the oral cavity with excellent diagnostic accuracy. For detecting carcinoma in situ or squamous cell carcinoma (SCC) versus non-cancer, sensitivity was 0.931 and specificity was 0.931; for detecting SCC versus 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 versus 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-invasive evaluation of tissue sites suspicious for high-grade dysplasia or cancer. Other studies have utilised 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.* 2008). 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.

_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—OCT, polarisation-sensitive OCT (PS-OCT), laser fluorescence (DIAGNOdent, KaVo), quantitative laser fluorescence (QLF), fibre-optic transillumination—exploit this concept, achieving varying degrees of specificity and sensitivity for detecting demineralisation and decay of the dental matrices, the anatomical structure of the tooth organ, as well as the attached microbial biofilms and calculus.

_Dental caries

A. 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, travelling 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 travelling at and the polarisation of the light. If the change of refraction between the media is gradual, the reflection will be minimal (Brenzinski *et al.* 2006; Colston *et al.* 1998; Feldchtein *et al.* 1998; Otis *et al.* 2000). The changes between the hard tissues such as enamel and dentine and between healthy and demineralised or carious states can then be interpreted to create 2-D and 3-D images of the hard tissues. As such, various optical properties are under investigation as potential quantifiers of the mineralisation changes to detect dental caries (Li *et al.* 2009). In the relatively early days of OCT, two groups of researchers investigated the feasibility of using OCT in vivo to image sound and demineralised tissue, and even monitored restorative procedures (Colston *et al.* 1998). A recent publication described the use of *in vivo* OCT to determine the effectiveness of a proton pump inhibitor in treating gastro-oesophageal reflux by monitoring dental erosion with OCT (Wilder-Smith *et al.* 2009). The study was significant in that the researchers were able to identify an association between the medication and a reduction in enamel erosion.

B. Polarisation-sensitive OCT

Since both enamel and dentine have strong polarising effects, changes in polarisation provide more structural information than conventional OCT (Brezinski, 2006). Light is delivered in one polarisation, and the reflection is read in both polarisations. Although we were unable to find clinical studies that used PS-OCT, extensive research has been conducted by Fried and others that demonstrates that this technology has the potential to monitor demineralisation/remineralisation and quantify demineralised tooth structure, even below dental sealant (Manesh *et al.* 2009; Chen *et al.* 2005; Jones *et al.* 2006; Jones & 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).

C. Laser fluorescence

Back-scattered light from laser-induced fluores-

Fig. 1_Indispensable part of a successful therapy: optical diagnostic.

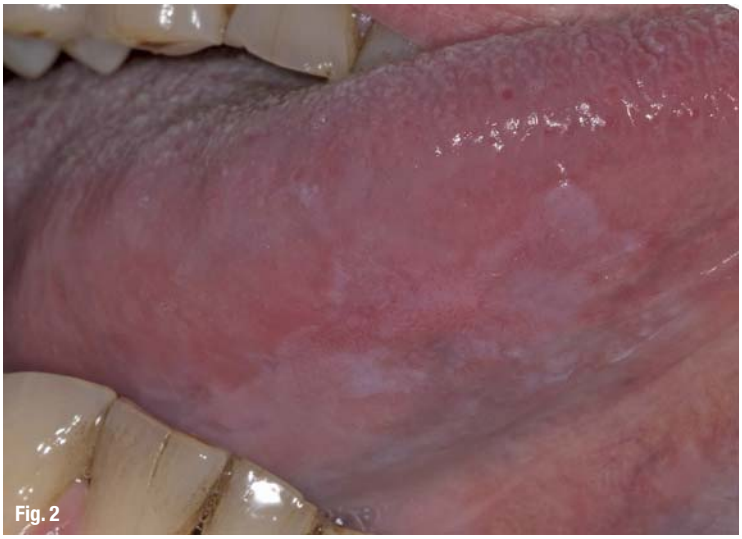

Fig. 2_Severe dysplasia under white light. (With kind permission of 14th Floor Solutions; VELscope®)

cence has been reported as a tool to detect and quantify caries activity (Zandona & 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 printout and an audible signal when decay is detected. The results of studies investigating diagnostic usefulness of DIAGNOdent vary considerably (Chong *et al.* 2003; Kuhnisch *et al.* 2008). The lack of diagnostic consistency may reflect: (a) the need for clinicians to learn how to use the correct position for the unit; (b) staining and/or calculus affecting the readings; and (c) difficulty in determining the numerical value at which surgical intervention is indicated (Shi *et al.* 2000). However, the literature appears 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). While 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.

D. Quantitative light fluorescence

QLF uses fluorescence induced by multi-wavelength excitation at 290 to 450 nm to measure mineral loss in enamel and dentine (Hall & Girkin 2004). Unlike the DIAGNOdent system, this device provides colour-coded images of the target tissue. Sound tooth structure fluoresces and carious tooth structure appears dark. 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 *et al.* 2004). In a high-risk population, QLF is highly predictive (.90–.98) of future caries (Zandon & Zero 2006). In a low-risk population, it is much less predictive, and stains, plaque, and fluorosis can affect QLF accuracy (Zandona & Zero 2006). High-intensity UV light can generate free radicals, potentially resulting in toxicity to live tissue.

E. Fibre-optic transillumination

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-imaging fibre-optic transillumination (DIFOTI), 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 overtreatment and is also unable to determine lesion depth, which limits potential sites of use (Young *et al.* 2005; Bin-Shuwaish *et al.* 2008; Schneiderman *et al.* 1997). Recently, Wu and Fried used near infra-red (NIR) transillumination to image dental caries (Wu & Fried 2009). This technology takes advantage of the transparency of sound enamel at 1310 nm, which decreases considerably in unhealthy tooth structure. Demineralised areas on the enamel surface appear lighter, while deeper lesions appear darker. However, low contrast as compared to the high reflectance signal and decreasing effectiveness with increasing tooth thickness are important clinical challenges. Although we were unable to identify clinical studies using NIR transillumination, the concept holds great promise, for example, allowing clinicians to monitor remineralisation of enamel.

_Other dental applications

Periodontics

A. Fluorescence using the periodontal 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 DIAGNOdent perio probe may aid in clinical detection of sub-gingival calculus deposits far better than conventional methods (Kasaj *et al.* 2008; Krause *et al.* 2003; Krause *et al.* 2005). 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.

B. 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 tissue, the enamel–cementum and the gingiva–tooth interfaces (Colston *et al.* 1998). While results of early *in vivo* studies were promising, consistent imaging of

the periodontal tissue remains challenging owing to the limited penetration depth and scan sizes of OCT (Colston *et al.* 1998). In another study by Baek *et al.* the successful use of OCT for monitoring periodontal ligament changes during orthodontic tooth movements in rats was reported (Baek *et al.* 2009).

Endodontics

A. Fluorescence using the DIAGNOdent perio probe

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, was 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 tissue and sound dentine were evaluated in 50 extracted teeth with known endodontic pathology. Sound dentine and healthy pulpal soft tissue gave an average fluorescence reading of 5 (on a scale of 100), whereas biofilms of *Enterococcus faecalis* and *Streptococcus mutans* colonising the root canals showed a progressive increase in fluorescence signals over time. 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 peri-apical pathology and scanning electron microscopy evidence of bacterial infection (Sainsbury *et al.* 2009).

B. 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 seconds for a 15 mm-long root. The authors concluded that fibre-optic OCT probing holds promise for full *in vivo* endodontic imaging.

Another *ex vivo* study assessed apical micro-leakage 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 peri-apical diagnostics is limited by its short penetration depth into the bone in which the tooth is embedded.

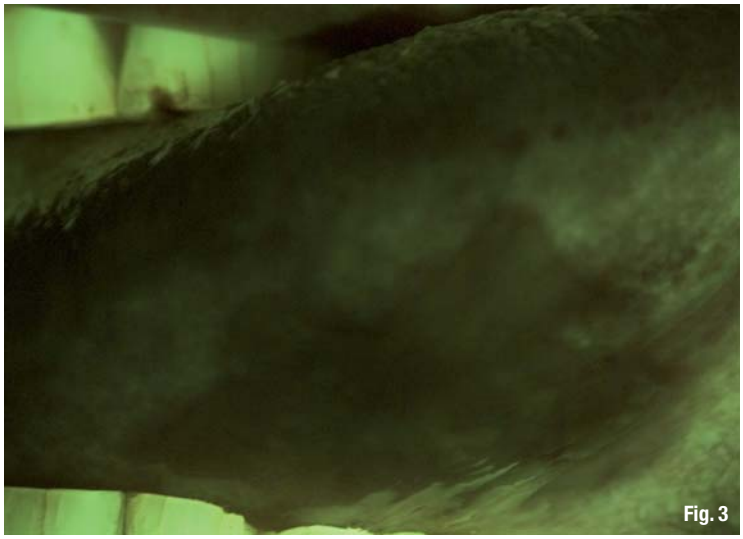

Fig. 3_The lesion viewed using VELscope's fluorescence visualization. (With kind permission of 14th Floor Solutions; VELscope®)

_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 are improving rapidly, such that many such devices are becoming available to dental clinicians. We envisage many benefits to patients and clinicians from the use of these devices._

_contact laser

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