Optical Coherence Tomography correlated with a functional fluorescence imaging for detection and quantification of dental caries

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ABSTRACT

Fluorescence radiance loss in enamel following demineralisation has been correlated to the amount of mineral lost during the demineralisation. The correlation between fluorescence loss measured by Quantitative Light-induced Fluorescence (QLF) and the reflectivity loss measured by an *en-face* Optical Coherence Tomography (OCT) system was investigated in a demineralisation process to produce artificial caries. We used an OCT system which can collect A-scans (reflectivity versus depth graph), B-scans (longitudinal images) and C-scans (*en-face* images). The power to the sample was 250 μW, wavelength λ = 850 nm and the depth resolution in air 16 μm. Transversal and longitudinal images showed the caries lesion as volumes of reduced reflectivity. A-scans, which show the profile of the reflectivity versus depth of penetration into the tooth tissue, were used for quantitative analysis of the reflectivity loss. Both the fluorescence radiance and reflectivity of the enamel decreased with increasing demineralisation time. A linear correlation was observed between the percentage fluorescence loss measured by QLF and the percentage reflectivity loss measured by OCT. It was concluded that the decrease in reflectivity of the enamel during demineralisation, measured by OCT, could be related to the amount of mineral lost during the demineralisation process

Key words: Optical coherence tomography, low coherence interferometry, quantitative light-induced fluorescence, confocal imaging, dental imaging, caries diagnosis, dental caries, demineralisation, enamel, optical methods

1. INTRODUCTION

Limited quantitative methods are available for clinical detection and measurement of tooth enamel demineralisation that produces dental caries. Quantitative analysis permits longitudinal assessment of the changes in the mineral status within the caries lesion, which would enable the effect of the advice and treatments tailored to inhibit demineralisation and promote remineralisation to be determined. Quantitative Light-induced Fluorescence (QLF) has been used for caries analysis[1], based on the established correlation between the mineral loss and the fluorescence radiance loss in enamel following demineralisation[2]. However, this system cannot show the depth of the demineralisation inside the tooth, which is essential for decision on the choice of treatment. Radiography[3] has been used for this decision-making for many years, but this technique exposes the patients to dangerous ionising radiation and, is non-quantitative. It seems appropriate, therefore, to develop a system that can detect and quantitatively monitor demineralisation without limitations.

Optical Coherence Tomography (OCT) has been used to produce longitudinal images of dental tissues[4-8] in which a reduction in enamel reflectivity was observed in areas of demineralisation[8]. It is envisaged that the decrease in reflectivity during demineralisation might be related to the amount of mineral lost. In the present study, a versatile *en-face* OCT system[9,10], developed initially for retina imaging, which can collect A-scan, longitudinal (B-scan) and *en-face* (C-scan) images of a biological tissue, was used to quantify the enamel reflectivity during a process of demineralisation

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to produce enamel caries. The correlation between the decrease of the enamel reflectivity measured by OCT and the loss in fluorescence radiance of enamel measured by QLF was determined.

2. METHODOLOGY

Fifteen freshly extracted bovine incisor teeth free from caries, cracks or enamel malformations were selected and polished with pumice slurry to remove organic contaminants from the labial surface. The teeth were then painted with two coats of a non-fluorescent acid-resistant colourless nail varnish, except for three exposed windows (W1,W2,W3) lying on the same horizontal level on the labial surface of the teeth. Caries-like lesions were then produced on each window by demineralisation of the teeth in acidic buffer solutions containing 2.2mM KH_2PO_4, 50mM acetic acid, 2.2mM of 1M $CaCl_2$ and 0.5ppm fluoride, at a pH of 4.5[11]. Prior to demineralisation (0 hour), the OCT (A, B, C) scans and fluorescent images of the windows on each tooth were recorded with the OCT and QLF systems respectively. OCT imaging was repeated on the windows W1, W2, and W3 at 24, 48, and 72 hours respectively. After imaging on each window, that window was sealed-off with adhesive tape to prevent further demineralisation until the last recording at 72 h on W3. Following this, the fluorescent image of each tooth was captured using the QLF clinical system and the lesions (Fig. 1A-D) were analysed quantitatively with QLF software, as described by Amaechi and Higham[1], to calculate the percentage fluorescent loss (ΔQ). Briefly, this is obtained by reconstructing the fluorescence radiance of sound enamel at the site of the lesion from the fluorescence radiance of the surrounding sound enamel (assumed to be 100%). The decrease in fluorescence was determined by calculating the percentage difference between actual and reconstructed fluorescence surface. Any area with a fluorescence radiance drop of more than 5% is considered to be lesion. The OCT images were acquired as described in previous publications[9,10]. Optical pathlength spectroscopy was used to calculate the degree of reflectivity (R) of the tissue at any depth, as described by Amaechi *et al.*[12]

3. RESULTS

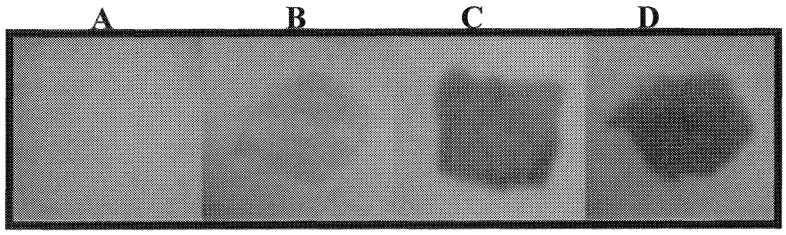

Fig. 1. QLF images of (A) sound tooth surface and demineralised (caries) surfaces; (B) 24 hrs, (C) 48 hrs and (D) 72 hrs demineralisation.

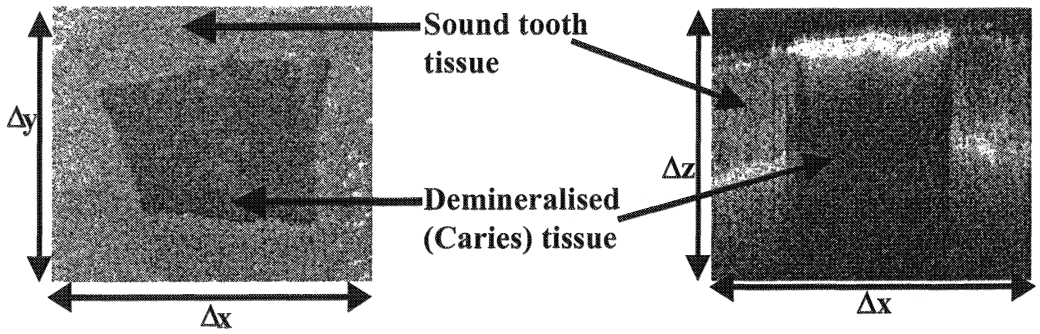

Fig. 2. Transversal OCT image of the lesion in figure 1D at a depth of 0.3 mm. Δ x = Δ y = 3 mm

Fig. 3. Longitudinal OCT image of the caries lesion in figure 1D showing its depth in the tooth tissue. Δ x = 3 mm, Δ z = 0.6 mm

While both transversal (Fig. 2) and longitudinal (Fig. 3) OCT images showed the caries lesion as volumes of reduced reflectivity, the longitudinal images additionally showed the depth of the lesion into the tooth tissue. The depth of the lesion into the tooth tissue was determined by correlating measurements obtained via both longitudinal and *en-face* OCT imaging. The A-scan graphs (Fig. 4) showed the levels of reflectivity (dB) versus depth (mm) of penetration into the tooth tissue. Quantitative analysis[12] of the degree of reflectivity of the tooth tissue evaluates an integral over the area below the reflectivity versus depth curve and results in a dB.mm value. Such analysis showed that the reflectivity of the tissue decreased with increasing demineralisation time (Figure 4 A, B); 0 hr=31.86±9.30 dB.mm, 24=14.45±5.47, 48=8.66±1.96, 72=4.32±2.72 (Pearson correlation (R Vs Time) coefficient, r = -0.944). There was a significant difference (ANOVA, α=0.05) between the mean values of R (n=15, $p<0.001$) at the 4 measurement intervals. For comparison with the QLF analysis data, the percentage reflectivity loss ($R_{\%}$) was calculated as follows:

$$\% \text{ Reflectivity loss (\%dB.mm)} = \frac{(R_{Sound} - R_{Demineralised}) \times 100}{R_{Sound}}$$

It was observed that the percentage reflectivity loss increased with increasing demineralisation time (Figure 5), and a linear correlation (r =0.963) was observed between the percentage fluorescence loss, ΔQ (%.mm^2) measured by QLF and the percentage reflectivity loss in demineralised tissue measured by OCT (Figure 6).

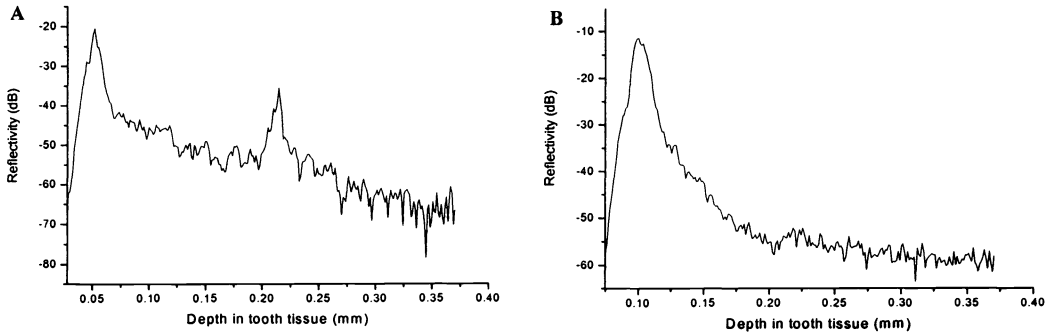

Fig. 4. A-scan showing the levels of reflectivity (dB) versus depth (mm) of penetration into the tooth tissue, and illustrating the decrease in reflectivity with demineralisation (A, B). A= Sound tooth tissue, B= Demineralised.

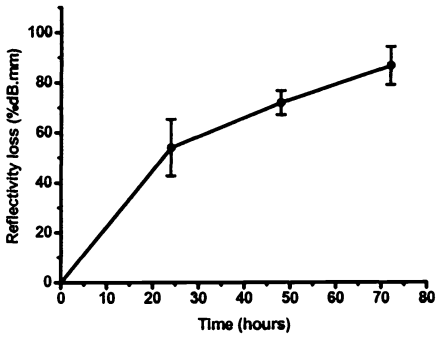

Fig. 5. Illustration of the increased % reflectivity loss with increasing demineralisation time as quantified using OCT.

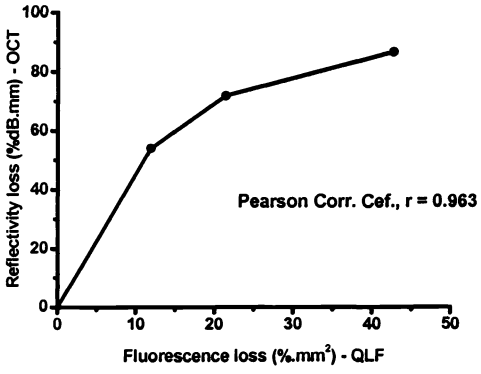

Fig. 6. Illustration of the linear correlation between the % reflectivity loss measured with OCT and the % fluorescence loss measured with QLF.

4. DISCUSION

The utilisation of OCT to detect an incipient caries lesion, as early as 24 hours of its development, was successfully demonstrated in the present study. OCT was able to discriminate between sound and demineralised (carious) tooth tissue by their degree of reflectivity; depicting carious tissue as volumes of reduced reflectivity (Figures 2 and 3). The system is presently being developed to use polarisation to discriminate between sound and demineralised tissue. In the present study, different levels (1-3 days) of demineralisation (and hence different levels of mineral loss) was shown by OCT as different degree of reflectivity loss, and in this way it was able to monitor the change in mineral status of the tooth tissue over a period of time. The decision to remineralise or restore a caries lesion depends on the depth of the lesion into the tooth. OCT was also able to show the depth of the demineralisation (caries) inside the tooth tissue with the longitudinal images (Figure 3), thereby proving to be a potential tool for decision-making in restorative dentistry. Amaechi *et al.*[12] have demonstrated that OCT A-scans deliver quantitative data relating to the degree of change in reflectivity, and hence the degree of change in mineral level, of the tooth tissue following development of caries. The provision of quantitative data as well as giving information regarding depth, all without dangerous ionising radiation, gives OCT superiority over the conventional x-ray technology which has been the tool for caries diagnosis for many years. This ability of OCT was validated in the present study with a method which has long been established for detection and quantification of early enamel demineralisation. QLF is an optical technique, which uses the natural fluorescence of teeth to discriminate between caries and sound enamel, based on the fact that the fluorescence radiance of a carious spot viewed with QLF is lower than that of surrounding sound enamel[1,2,13]. QLF measures the percentage fluorescence radiance change of demineralised enamel with respect to surrounding sound enamel, and relates it directly to the amount of mineral lost during demineralisation, though it does not show the depth of the lesion. However, the good correlation between OCT and an established method of detection and quantifying demineralisation signified that the reflectivity lost in enamel during demineralisation, measured using OCT, could be related to the amount of mineral lost during the demineralisation process.

OCT is an imaging modality, which was developed mainly on two reasons: (i) based on interference, low level signals reflected by the tissue are enhanced by the strong signal power in the reference arm of the interferometer; (ii) using a source with low coherence length, depth resolved images can be produced with good rejection of multiply scattered light (depth sampling interval is 10 - 20 μm when using superluminiscent diodes (SLD) and could be below 4 μm when using more expensive Kerr lens mode-locked lasers[14]. The OCT technique applied to ophthalmology has evolved rapidly in the last few years[15]. Applications of OCT in dentistry have already been reported, covering *in vitro* images of dental and periodontal tissues[4,5] as well as carious lesions[6,7,8]. However, all the reported methods delivered only longitudinal OCT images, which we believe, restricts the interpretation of the high-resolution images. The system used in the present study, which is based on confocal microscopy and low coherence interferometry[9,10], can operate in different regimes to deliver both longitudinal and transversal images[16] (Fig. 2 and 3). The apparatus comprises an interferometer excited by a pigtailed superluminiscent diode (SLD), central wavelength $\lambda = 0.85$ μm, bandwidth $\Delta\lambda = 20$ nm which sends 250 μW power to the tooth. In the sensing arm of the OCT, a splitting device redirects a part of the reflected light from the tooth towards a photodetector, behind a lens and a pinhole, used in the confocal receiver.

Any OCT is built around a confocal microscope. However, in our system[9,10] a separate confocal channel is provided. The confocal image was useful for identifying the lesions, aligning the tooth and evaluating the overall map of reflectivity along X or Y axis in the longitudinal regime or in the plane (x,y) in the transversal regime. The confocal channel may also be very useful *in vivo*, when lateral movement of the patient in the scanning direction X or Y respectively picked up from the confocal image can be used to correct the OCT image.

When in transversal regime, en-face images collected at different depths (42 pair-frames from a volume in depth of 1 mm in air were acquired in 20 s in present study) are subsequently used to reconstruct 3D volumes of the tissue. The reconstruction allows software inferred OCT longitudinal images at any transversal position in the stack. The position in depth in the stack before creating longitudinal OCT images is also adjustable, offering a valuable guidance tool for exploring the 3D volume of the tissue. The system, equipped with the 3D rendering feature acts as a valuable diagnostic tool allowing "peeling off" of transversal and longitudinal biologic material to investigate different internal features. The

confocal image can also be displayed sideways, along with the *en-face* OCT image at each depth. The software allows the reconstruction of the 3D profile to be seen from different angles. Successive displays of transversal and longitudinal cuts at different positions in the 3D stack of en-face OCT images give a direct view of the caries volume. The 3D imaging mode also helped in choosing the position of the A-scan in transversal section.

5. CONCLUSION

It was concluded that OCT, as a technique for detection and analysis of early enamel caries, correlated well with an established method of quantifying demineralisation. The reflectivity lost in enamel during demineralisation, measured using OCT, could be related to the amount of mineral lost during the demineralisation process. OCT detected early enamel caries, showed the depth of the caries inside the tooth tissue, and quantitatively monitored tooth tissue demineralisation over a period of time. This demonstrated that OCT would be a suitable tool for routine examination in the dental clinic, and a useful device to quantitatively monitor, *in vivo,* the mineral changes over time in a caries lesion on application of a therapeutic agent. It would also be applicable in an *in vivo, in situ* or *in vitro* testing of the efficacy of products formulated to inhibit demineralisation and/or promote remineralisation. The potential of OCT as a caries diagnostic device, which may possibly replace the conventional dental radiograph to eliminate the danger of hazardous ionising radiation, was highlighted by the present study.

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