Quantification of Root Caries using Optical Coherence Tomography and Microradiography: a Correlational Study

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Purpose: The use of transverse microradiography (TMR) to quantify the amount of mineral lost during demineralization of tooth tissue has long been established. In the present study, the use of an *en-face* Optical Coherence Tomography (OCT) technology to detect and quantitatively monitor the mineral changes in root caries was investigated and correlated with TMR.

Materials and Methods: We used an OCT system, developed initially for retina imaging, and which can collect A-scans, B-scans (longitudinal images) and C-scans (*en-face* images) to quantitatively assess the development of root caries. The power to the sample was 250 μW, wavelength $\lambda = 850$ nm and the optical source linewidth was 16 μm.

Results: Both the transversal and longitudinal images showed the caries lesion as volumes of reduced reflectivity. Quantitative analysis using the A-scan (reflectivity versus depth curve) showed that the tissue reflectivity decreased with increasing demineralization time. A linear correlation ($r = 0.957$) was observed between the mineral loss measured by TMR and the percentage reflectivity loss in demineralized tissue measured by OCT.

Conclusion: We concluded that OCT could be used to detect incipient root caries, and that the reflectivity loss in root tissue during demineralization, measured by OCT, could be related to the amount of mineral lost during the demineralization.

Key words: optical coherence tomography, root caries, confocal imaging, dentin caries, quantitative transverse microradiography

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The incidence of root caries is increasing as a result of increase in the number of people retaining their natural dentition in old age, consequent to improvements in oral health care and more people living longer (Katz, 1981). Although the mechanism of root caries is the same biologically and chemically as enamel caries, demineralization progresses more rapidly and to a greater extent than a lesion in enamel because less mineral is present, and once demineralized, the organic matrix becomes more susceptible to bacterial enzymatic degradation, thus easily exposing the pulp to the risk of bacterial invasion (Wefel, 1994). In addition, because root caries involves only cementum and/or dentin it is more challenging to treat both by chemical and conventional restorative techniques, due to lack of enamel for resin-based bonding which limits the effectiveness of these more esthetic sys-

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tems. Because root caries is more hazardous to pulpal health and restoration is difficult, aggressive prevention is recommended. From a prevention-based dentistry perspective, the best strategy is early detection and early intervention. Early detection of root caries would permit the implementation of remineralizing therapy (such as fluoride application) (Jones, 1995; Brailsford et al, 2002), which is more cost effective, and less time consuming compared to a restorative procedure. However, because of the damaging effects of explorer, the primary detection method must be a discriminating eye. Unfortunately, the detection of early root (dentin) caries, by visual examination, has been a problem in clinical dentistry owing to the fact that early dentin caries unlike enamel caries does not present as white spot lesion. Fluorescent dyes have been used to aid clinical detection of root caries, by either visual examination (Wilkinson et al, 1997) or optical technologies (van der Veen et al, 1996; Amaechi and Higham, 2002). However, most of these dyes either stain both the sound and carious tissue or are not clinically approved for intra-oral use. Thus, there is still a need to develop an appropriate method for early clinical detection. In addition to detection, the new technology should be capable of quantification of the demineralization that produces root caries. Besides permitting longitudinal assessment and monitoring of the changes in the mineral status within the caries lesion, quantitative analysis would make it possible to determine the effect of the advice and treatments tailored to inhibit demineralization and promote remineralization.

Optical Coherence Tomography (OCT) is an imaging technique which has been shown to provide high resolution images of dental tissues including caries lesions in enamel (Baumgartner et al, 1998, 2000; Colston et al, 1998a,b; Feldchtein et al, 1998). However, all previous reports refer to only longitudinal OCT imaging without quantitative analysis. Furthermore, the information which can be collected from longitudinal images is obviously limited. It would be more natural to see *en-face* slices in the tooth in the way we are used to see them when looking through a microscope. Therefore, in the present study, we used an *en-face* OCT technology, developed initially for retina imaging (Podoleanu and Jackson, 1998; Podoleanu et al, 1998), to determine the application of the OCT into detection and quantification of root caries, and to correlate it with transverse microradiography (TMR), a method that has long been established as a gold standard for quantification of demineralization of tooth tissue (de Josselin de Jong et al, 1987; Amaechi et al, 1998a).

MATERIALS AND METHODS

Caries Production and OCT/Confocal Imaging

Roots of thirty extracted human molar teeth were collected, cleaned of all debris and soft tissue and examined. Those roots that were free from caries, cracks or enamel malformations were selected. The roots were then painted with two coats of acid-resistant nail varnish, except for three exposed windows (D1, D2, D3), each measuring approximately 2 mm in diameter, lying on the same horizontal level on the same surface of the root. Caries-like lesions were then produced on each window by demineralization 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 (Amaechi et al, 1998b). Prior to demineralization (0 hour), the OCT (A, B, C) scans of the windows on each root were recorded. OCT imaging was repeated on the windows D1, D2, and D3 at 24, 48, and 72 hours respectively. After imaging on each window, that window was sealed-off with adhesive tape to prevent further demineralization until the last recording at 72 h on D3. The OCT images were acquired as described in previous publications (Podoleanu and Jackson, 1998; Podoleanu et al, 1998; Amaechi et al, 2003a,b). The A-scans, obtained by optical pathlength spectroscopy, which showed the depth (mm) resolved reflectivity (dB) of the tooth tissue, were used to calculate the degree of reflectivity, R (dB.mm), of the tissue at any depth in accordance with the method described by Amaechi et al (2001).

Quantitative Transverse Microradiographic Image Analysis

Following OCT imaging, root sections of approximately 130 µm thick were cut from each window, using a water-cooled hard tissue microtome (Precise Corp. Wisconsin, USA). The sections were then mounted on brass anvils with nail varnish, and polished to give planoparallel specimens of 80 µm thickness using a diamond disc. Following this, the

sections were mounted on a microradiographic plate-holder bearing an aluminum stepwedge (Ten 25 μm steps).

Together with the stepwedge, the microradiographs of the sections were taken by a 10-minute exposure on Kodak high-resolution plates (Type 1A) using a Cu(Kα) X-ray source (Philips BV, Eindhoven, The Netherlands) operating at 20 Kv and 10 mA at a focus-specimen distance of 30 cm. The plates were developed using standard techniques. The microradiographs were then subjected to examination and image analysis, using a Leica Leitz DMRB optical microscope (Leica, Wetzlar, Germany). The image (Fig 1) was captured at a magnification of 20x/0.40 via a Sony model XC-75CE CCTV camera (Sony, Tokyo, Japan) connected to a computer (Viglen PC, London, UK), and analyzed under standard conditions of light intensity and magnification and processed, along with data from measurement of the stepwedge, to quantify the mineral loss, ΔZ (Vol %.μm), using a software package (TMRW v.1.22, Inspektor Research System BV, Amsterdam, The Netherlands) based on the work described by de Josselin de Jong et al (1987).

Statistical Analysis

The data obtained was analyzed statistically with the significance level (α) prechosen at 0.05. In order to assess the ability of OCT to quantitatively monitor the mineral changes in a tooth tissue during demineralization, the mean values of the degree of reflectivity, R (dB.mm), of the tooth tissue at the 4 measurement intervals, 0, 24, 48 and 72 hours were compared using analysis of variance (ANOVA) for repeated measures, and also correlated with time using Pearson correlation coefficient (r). For comparison with the mineral loss measured by TMR, the percentage reflectivity loss ($R_{\%}$) was calculated as follows:

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

The correlation between $R_{\%}$ and ΔZ was determined using Pearson correlation coefficient (r), while coefficient of linear regression was used to establish an equation tying the two measurement quantities.

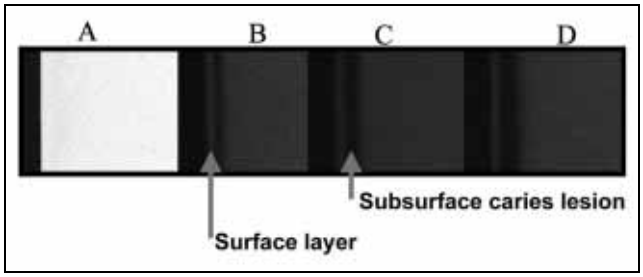

Fig 1 Microradiographic images of root (dentin) sections from (A) sound root surface and demineralized (caries) surfaces: (B) 24 hrs, (C) 48 hrs and (D) 72 hrs demineralization showing subsurface lesions.

RESULTS

The 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 A-scan graphs (Fig 4) showed the levels of reflectivity (dB) versus depth (mm) of penetration into the tooth tissue. Quantitative analysis (Amaechi et al, 2001) of the degree of reflectivity of the tooth tissue evaluates an integral over the area below the reflectivity versus depth curve (Fig 4) and results in a dB.mm value. This analysis showed that the degree of reflectivity, R (dB.mm) decreased with increasing demineralization time: 0 hr = 16.67 ± 2.66 dB.mm; 24 = 12.80 ± 3.83; 48 = 9.16 ± 1.54; 72 = 7.46 ± 1.65; and this was confirmed by Pearson correlation coefficient (R Vs time, r = – 0.957). The mean R values (n = 30) at the 4 measurement intervals were significantly different (ANOVA, $p < 0.01$). It was observed that $R_{\%}$ increased with increasing demineralization time, demonstrating a similarity with the mineral loss, ΔZ (Fig 5). A good correlation was indicated between $R_{\%}$ and ΔZ by both Pearson correlation coefficient (r = 0.987) and coefficient of linear regression ($R_{\%} = -0.66 + 0.05\Delta Z$, $r^2 = 0.97$).

DISCUSSION

OCT is a new imaging modality, which could non-invasively, image the internal microstructures of biological tissues such as tooth (Baumgartner et al, 1998; Baumgartner et al, 2000; Colston et al, 1998a, b; Feldchtein et al, 1998), eye (Podoleanu and Jackson, 1998; Podoleanu et al, 1998) and skin (Podoleanu et al, 2000). It is based on confo-

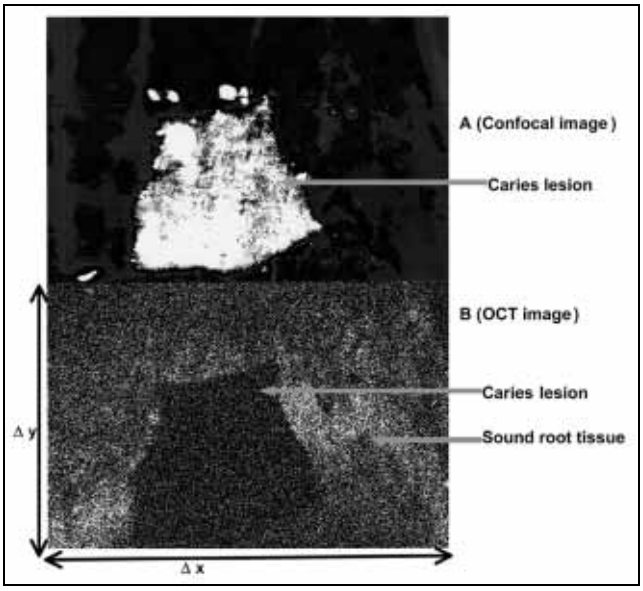

Fig 2 Confocal microscope image of the caries lesion in Fig. 1D displayed alongside the transversal (*en-face*) OCT image of the lesion at a depth of 0.3 mm. $\Delta x = \Delta y = 3$ mm. The caries appeared as bright in confocal image.

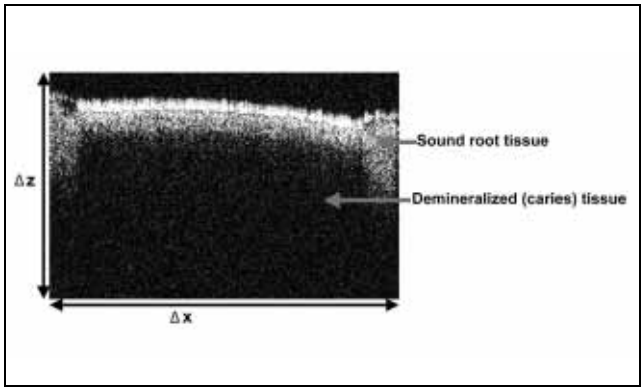

Fig 3 Longitudinal OCT image of the root caries lesion in Fig. 1D showing its depth in the tooth tissue. $\Delta x = 3$ mm, $\Delta z = 0.6$ mm.

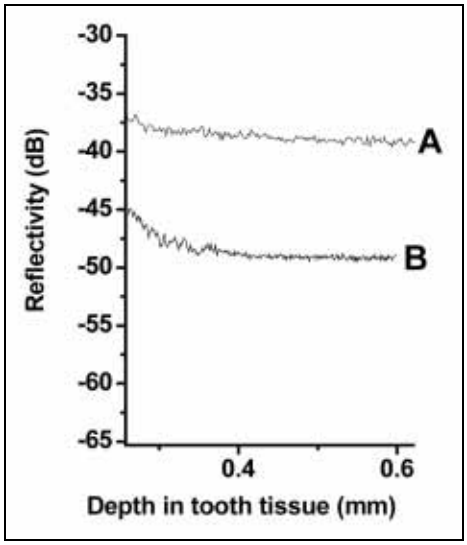

Fig 4 A-scan taken from the middle of the OCT image in Fig. 2, showing the levels of reflectivity (dB) versus depth (mm) of penetration into the tooth tissue, and illustrating the decrease in reflectivity with demineralization (A, B). A = Sound tooth tissue, B = Demineralized.

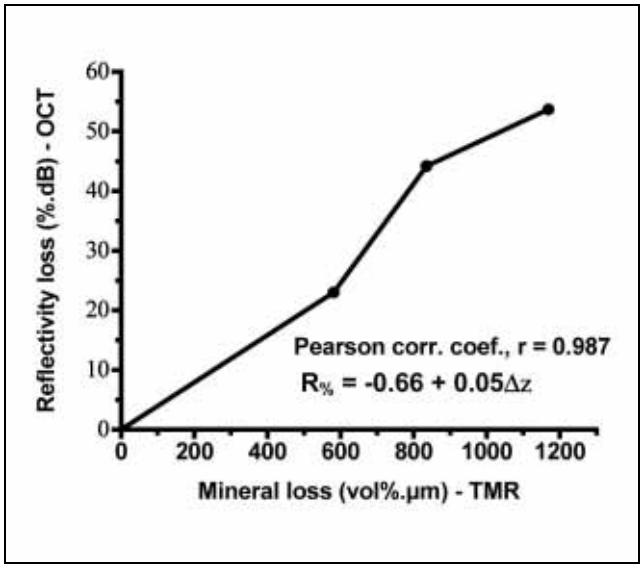

Fig 5 Illustration of the linear correlation and the relationship ($R_{\%} = -0.66 + 0.05\Delta z$) between the percentage reflectivity loss ($R_{\%}$) measured with OCT and the mineral loss (Δz) measured with TMR.

cal microscopy and low coherence interferometry. Based on the principle that the highest quality image information is contained in the portion of the detected light that is relatively unscattered and therefore travels the most direct path through the tissue, OCT uses low coherence interferometry to selectively remove the component of backscattered signal that has been multiply scattered, resulting in very high resolution images.

The present study demonstrated that OCT can perform high resolution cross sectional imaging of the internal structure of the tooth *in vitro* and in real time, and it is believed that it can do same *in vivo* and *in situ*. Applications of OCT in dentistry have al-

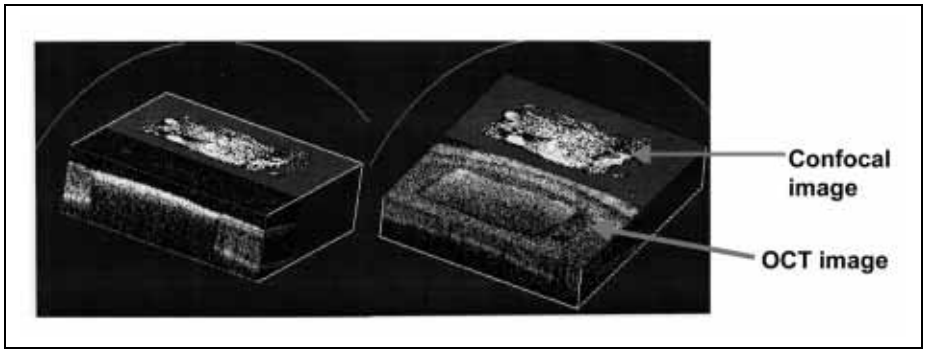

Fig 6 Stack of 42 pairs of OCT and confocal images viewed at different depths, as indicated below each frame. Lateral size: (5 mm x 5 mm).

ready been reported, covering *in vitro* images of dental tissues (Colston et al, 1998a) and of caries lesions (Baumgartner et al, 2000). However, all the reported methods required long acquisition times (25–45 seconds a frame (Colston et al, 1998b)) and delivered only longitudinal OCT images, which we believe, restricts the interpretation of the high-resolution images. The system used in the present study can operate in different regimes to deliver both longitudinal and transversal images (Figs 2 and 3).

In the longitudinal regime, the depth range was 1 mm in air for the images presented in this paper. The generation of B-scan images differs from other reports on longitudinal OCT imaging (Huang et al, 1991) where the B-scan image is constructed out of A-scans (depth profiles of the reflectivity in depth). The longitudinal images showed the depth of the demineralization (caries) inside the tooth tissue (Fig 3), thereby proving OCT to be a potential tool for decision-making in restorative dentistry since the decision to remineralize or restore a caries lesion depends on the depth of the lesion into the tooth. The *en-face* images are obtained in the transversal regime and the depth interval between successive C-scans were about 25 μm. In this way, 40 pair-frames from a volume in depth of 1 mm in air were acquired in 20 s in the present study. *En-face* images collected at different depths are subsequently used to reconstruct 3D volumes of the tissue (Fig 6). The same software developed by S. Dunne and used before to produce 3D images from the retina and skin is used (Podoleanu et al, 2000). 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. This is illustrated by movies showing either depth or lateral exploration along one of two possible different directions in the stack of transversal OCT images. 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 (Fig 2). The confocal image is useful in identifying the caries lesion and aligning the tooth. This would be very useful for *in vivo* application of the system. The use of A-scan from OCT imaging to produce a 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 has been demonstrated (Amaechi et al, 2001). The provision of quantitative data as well as giving information regarding depth, all without dangerous ionizing radiation, gives OCT superiority over the conventional X-ray technology which has been the tool for caries diagnosis for many years.

The ability 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 demineralized (carious) tooth tissue by their level of reflectivity; depicting carious tissue as volumes of reduced reflectivity (Figs. 2 and 3). The system is presently being developed to use reflectivity, polarization and birefringence to discriminate between sound and demineralized tissue. In the present study, different levels (1–3 days) of demineralization (and hence different levels of mineral loss) was shown by OCT as varied degree of reflectivity loss, and thus demonstrating its ability to monitor the change in mineral status of the tooth tissue over a period of time. This showed that OCT would not only be a suitable tool for routine examination in the dental clinic, but would be 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 demineralization and/or promote remineralization.

The demonstrated ability of OCT to detect and quantify caries was correlated in the present study with a method that have long been established for quantifying demineralization of tooth tissue, quantitative transverse microradiography. TMR is an X-ray system, which has long been established as a laboratory method of quantifying the mineral loss in tooth tissue (de Josselin de Jong et al, 1987; Amaechi et al, 1998a), and is currently regarded as a gold standard for measuring the mineral content in a hard tissue.

CONCLUSION

It was concluded that OCT, as a technique for detection and analysis of early root caries, correlated well with an established method of quantifying demineralization. Therefore, the reflectivity lost in enamel during demineralization, measured using OCT, could be related to the amount of mineral lost during the demineralization process. OCT detected early root caries, showed the depth of the caries inside the tooth tissue, and quantitatively monitored tooth tissue demineralization over a period of time. This demonstrated that, when fully developed, the system would be a caries diagnostic device, which may possibly replace the conventional dental radiograph to eliminate the danger of hazardous ionizing radiation.

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