DOI: 10.1111/j.1365-263X.2009.01025.x

Characterization of enamel in primary teeth by optical coherence tomography for assessment of dental caries

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International Journal of Paediatric Dentistry 2010; 20: 158–164

Background. Caries is a disease that affects both primary and permanent dentitions, therefore new methods of caries diagnosis need to be tested on primary teeth as well as on permanent teeth.
Aim. This study reports the application of optical coherence tomography (OCT) to characterize sound dental structure and detect natural caries of human primary teeth.
Design. Six primary teeth were sectioned into thin slices (~1.5 mm), and analysed perpendicular to the enamel surface by two home-made OCT systems operating around 1280 and 840 nm. The generated images were compared with histology as the gold standard.
Results. We demonstrated the efficacy of the OCT technique to measure the depth of the enamel layer, whose result was statistically compared with histology ($P = 0.823$; $P > 0.05$) with good agreement. We also determined, through measurement of contrast values, an increase in backscattered intensity of the order of two to three times between sound and caries regions.
Conclusions. We employed OCT generated images to characterize the enamel layer. The technique showed great potential to be used on paediatric dentistry clinical on early caries detection with no pain, as it is a noninvasive method.

Introduction

Optical coherence tomography (OCT) is a nondestructive imaging technique that uses low-coherence interferometry to determine the echo time delay and magnitude of backscattered light reflected from a transparent or semitransparent structure[1]. The technique produces two (2D)- or three-dimensional (3D) images and is capable of measuring backscattered light as a function of optical depth up to 2–3 mm (depending on the type of tissue). OCT has been widely used to study bio-tissues, to characterize and detect alterations of the optical properties with axial spatial resolution ranging from 1 to 15 μm[2]. The state-of-the-art in spatial resolution is 0.5 μm, allowing imaging at the cellular level[3]. In dentistry, the first applications were reported in 1998[4], and as then it has been used for various applications, such as recurrent caries detection and marginal adaptation of restorations[5], characterization of periodontal structures[6,7], and early detection of oral cancer[8]. More recently, early caries diagnostics[9], and the analysis of the performance of dental materials[10,11] have been studied and reported by our group.

A basic OCT set-up consists of a broadband light source, whose spectral width determines the axial spatial resolution, while penetration depth is determined by the central wavelength due to absorption and scattering properties of the tissue; an interferometer, which generally employs a Michelson design containing the sample in one of the arms and a delay line in the other arm; and an optical detector, whose signal output is electronically treated and input to a computer for image generation. Additionally, most OCT techniques described for imaging of dental tissue have used super luminescent diodes as the light source with wavelengths from 840 to 1310 nm, as they can provide spectral bandwidth wide enough to support an axial resolution of ~10 μm with a very good beam quality, as well as being much more economical than a femtosecond laser source.

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As with any diagnosis technique for optical caries, it is necessary to understand the optical properties of dental hard tissue inherent to the complex, inhomogeneous biological structure. Dental enamel is an ordered array of inorganic apatite-like crystals surrounded by a protein/lipid/water matrix[12]. The crystals are clustered together and roughly perpendicular to the tooth surface. Due to this structure scattering distributions are generally anisotropic and depend on tissue orientation relative to the irradiating light source[13–15] in addition to the polarization of the incident light.

The near-IR region from 780 to 1550 nm offers the greatest potential for new optical imaging modalities due to the weak scattering and absorption in dental hard tissue[16]. The magnitude of light scattering in dental enamel is expected to decrease following a $1/\lambda^4$ law, λ being the source wavelength, due to the size of the principal scatterers in enamel[12]. By analysing the optical properties of carious enamel one can establish a method for characterizing the severity of the carious lesion. Demineralized regions present higher porosity, which in turn increases light backscattering, thus making the lesion appear brighter[17–19].

Despite the differences in structure and composition between permanent and primary teeth, research on early caries detection by optical systems in paediatric dentistry clinical is still required worldwide. As caries progresses more rapidly in primary enamel than in permanent enamel, new methods of caries diagnosis need to be tested on primary teeth as well[20].

Optical coherence tomography is promising for the quantification of mineral loss from dental caries but has only been thoroughly tested on permanent dentition. The objective of this study was to show a characterization of enamel layer structure, as well as detect and quantify mineral loss from natural carious lesions, using a spectral domain (SD) and a time domain (TD)-OCT technique and two different wavelengths.

Materials and methods

The experimental study was carried out after approval by the Ethical Committee (268/2007) of the Center of Health Sciences, Universidade Federal de Pernambuco, Brazil, in accordance with the ethical guidelines in research with human participants.

In this research four incisor and two canine primary teeth, lost physiologically, were collected and stored in physiological saline (0.9%) to preserve tissue hydration. All of them were transversally sectioned resulting in three layers (~1.5 mm), by a low speed diamond wheel saw, model 650 (SBT, Inc.), with irrigation.

To perform OCT imaging of teeth, two home-made OCT systems were explored: a set-up working in the TD-OCT and another one working in the SD-OCT, which schematic set-up are shown on Fig. 1a and b, respectively. The TD-OCT system operating at a central wavelength of 1280 nm, with a maximum average power of 5 mW, generated by a superluminescent diode (model SLD-571; SUPERLUM, Moscow, Russia), with a 64.6 nm bandwidth, which represents an axial resolution of 11 μm. An All-fibre beam splitter is used, and the delay line is a Fourier-domain delay line consisting of a grating and a scanning galvo. The incident beam was focused on the sample using an achromatic 50 mm focal distance lens. For a typical beam diameter of 1 mm before the focusing lens, the lateral resolution was ~80 μm. This optical delivery system is mounted in a 2D translation stage, which is then scanned in X (or Y) position for data acquisition. The recombined beams are fed into a photodetector and associated electronics, and the output is sent to a personal computer with a LabView based image construction.

The original TD-OCT method encoded the location of each reflection in time by relating the position of a moving reference mirror to the location of the reflection. The SD-OCT acquires all information in a single axial scan through the tissue by simultaneously evaluating the frequency spectrum of the interference between the reflected light and a stationary reference mirror.

Our home-made SD-OCT system operates at a center wavelength of 840 nm. The broadband source is a superluminescent diode (Broadband SLD Lightsource S840; SUPERLUM) delivering up to 25 mW and with

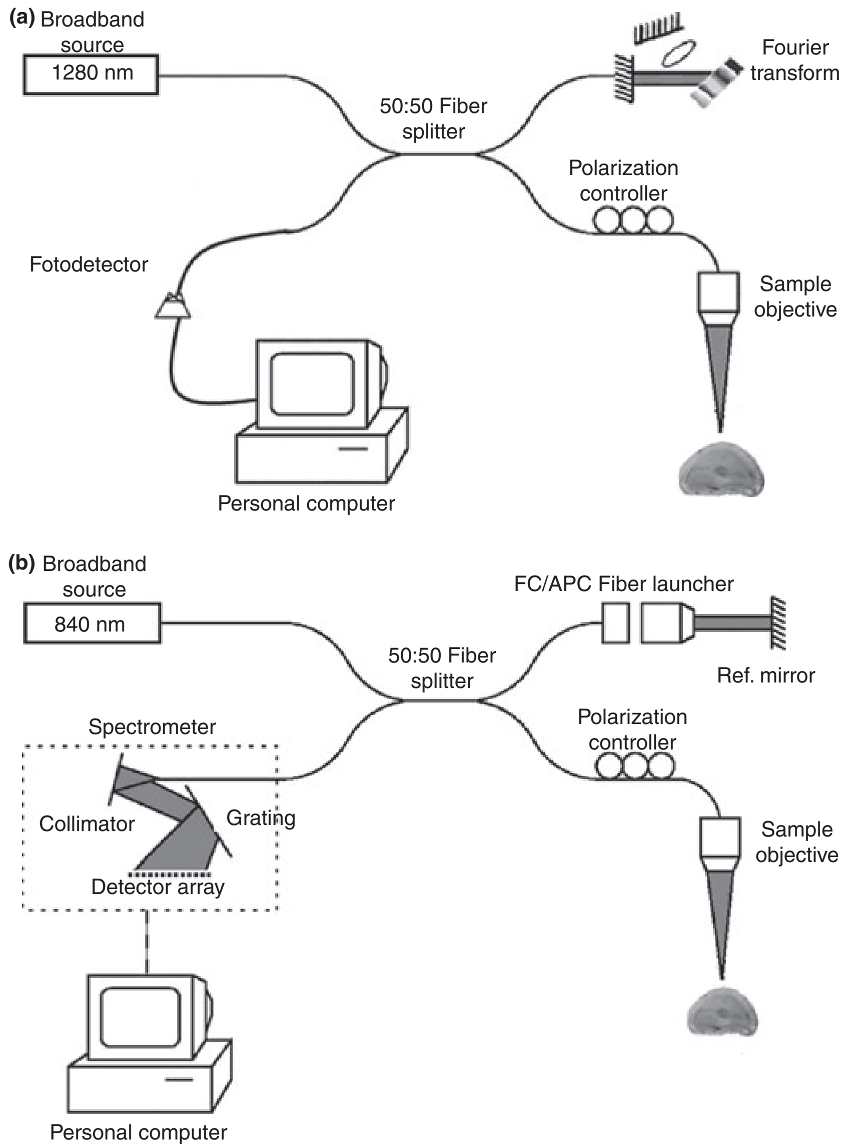

Fig. 1. Schematic diagram of the optical coherence tomography system. (a) TD-OCT operating at 1280 nm with a Fourier-domain delay line, (b) SD-OCT operating at 840 nm.

a 49.9 nm bandwidth, which gives an axial resolution of 6 μm. After travelling through the all-fibre beam splitter, the reflected beams from the sample and mirror are recombined and sent through a custom designed spectrometer consisting of a lens collimator system, a 1200 l/mm grating and a CCD (ATMEL, 2048 pixels, 12 bits, CA, USA). The maximum incident power on the sample was approximately 5 mW. The incident beam was focused on the sample using an achromatic 50 mm focal distance lens. For a typical beam diameter of 1 mm in front of the focusing lens, the lateral resolution was ~40 μm. This optical delivery system is mounted in a 2D translation stage, but the beam goes through a scanning galvo, which scans in *X* or *Y* for data acquisition, and therefore there are no movable parts. The output is sent to a personal computer with a LabView based image construction.

All images of the enamel slices were taken by scanning the enamel surface on all four faces (vestibule, lingual, mesial and distal). The light penetrated into the tooth structure and a tomographic image of the frame, perpendicular to the axis of tooth, was obtained. After the image construction by TD-OCT and SD-OCT, histological images of all samples were performed from both sides of the thickness using a conventional optical microscope coupled to a CCD camera. Measurements were done with a downloadable image processing program, ImageJ (NIH, MD, USA). The enamel thickness was measured in both techniques and values were compared by a correlation coefficient test. Based upon the structure of sound enamel, we easily observed small alterations of white spot demineralization, confirmed by histological evaluation.

Results

For comparison purposes, we first show, in Fig. 2a–c images obtained by optical microscope of three sections: cervical, medial, and incisal, respectively, of one of the studied samples. The dimensions of each section are shown by the scales.

Then all faces of each sample were examined by OCT and, as expected, because of the irregularity of the dimensions of the teeth, measurements of the enamel primary layer ranged from 150 to 1000 μm. In Fig. 3a–c, it is possible to compare both OCT images and histological images, and also identify with precision the enamel layer (E), the enamel–dentin junction and a thin layer of dentin (D) in all samples examined. The structures in the OCT images are distinguished by the different grey levels (or blue levels, in the case of the 850 nm system), where the limits of enamel layers appear whiter (highest scattered intensities) and dentin appears darker (lowest scattered intensity).

Notice that in the OCT measurements the value shown on the scale must be divided by the index of refraction of enamel ~1.62, to obtain the correct dimension[4]. Analysing images of the same area made by both OCT systems, it is possible to see a better resolution at 840 nm in the SD-OCT (due to the shorter wavelength) but a deeper penetration of light in the TD-OCT image operating at 1280 nm (mainly due to reduced scattering and absorption).

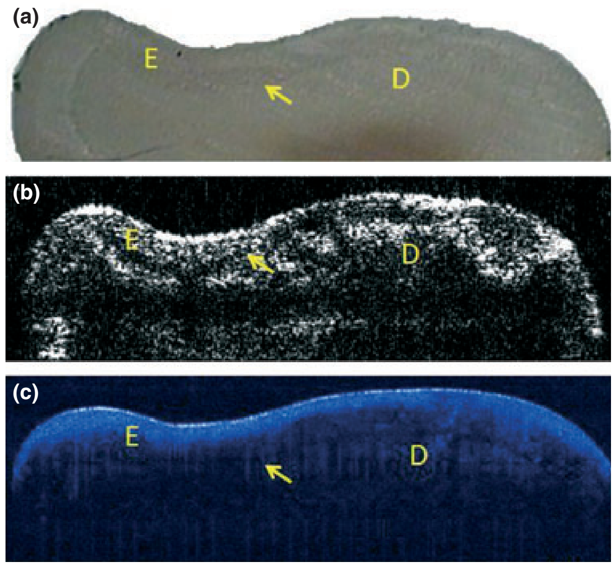

Fig. 3. (a) Enamel (E), dentine (D) and enamel–dentin junction (EDJ; indicated by the arrow) corresponding to the same lingual face of a primary sample, by histological imaging; (b) OCT image at 1300 nm; and (c) OCT image at 840 nm.

In Fig. 4a–c, a layout of OCT images is shown, which was constructed by superimposing OCT scans of different faces of the tooth section. It is possible, from Fig. 4b and c, to observe the enamel structure (superficial layer) and also a thin enamel crack (indicated by the arrow) that was clearly delineated due to the strong birefringence, while the dentin, due to the anisotropic light propagation through dentinal tubules, makes a diffuse image, backscattering light in very different directions.

In order to confirm statistically the precision measurements of each OCT system, we carried out a series of measurements of $n = 72$ enamel layers for each imaging system, evaluated by the ImageJ Software, whose results are shown

Fig. 2. Optical microscope image of three thickness of one primary tooth. (a) Incisal, (b) medial, and (c) cervical sections.

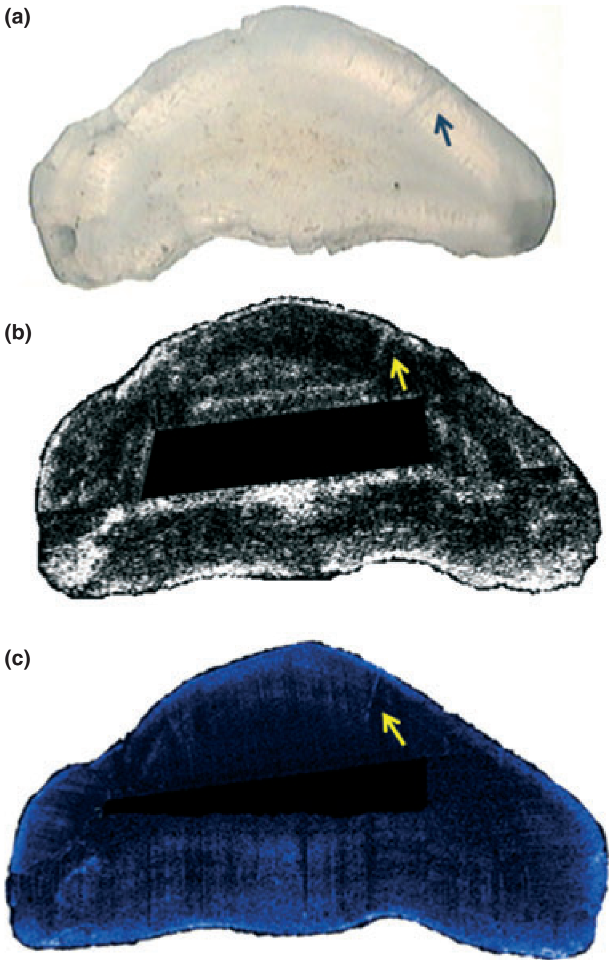

Fig. 4. Enamel structures (superficial layer) and thin enamel crack (indicated by the arrow). (a) Histological, (b) layout OCT 1280 nm and (c) layout OCT 840 nm. The OCT images were constructed by superimposing OCT scans of different faces of the tooth section.

in Table 1. The mean and standard deviation behaved in the same way, which was corroborated by a statistical correlation index test, indicating that both OCT systems were calibrated and effective for examining the enamel layer with precision.

Optical coherence tomography clearly demonstrates the capacity of quantitative assessment of the enamel and dentin of primary tooth, compared with the optical microscope. The deeper penetration depth of the light at 1280 nm compared to 840 nm is due to a large reduction of absorption and scattering coefficients of the enamel and dentin at longer wavelength[22].

As a final example, Fig. 5 shows a superposition of the four faces of one tooth section sample, but differently than in Fig. 4, the tooth presents caries decay. In Fig. 5a, the circle indicates the lesioned region, which is identified by the increased scattering. By using the ImageJ program, the contrast between the lesioned and healthy enamel is measured, as shown in Fig. 5b. In the grey scale, represented in the *Y*-axis in arbitrary units, the healthy enamel is the background with values below 0.05, whereas in the lesioned region the values approach 0.15. Therefore, it is possible to determine, through the contrast value, an increase in backscattered intensity of the order of two to three times, which corroborates early results by Huynh *et al.*[21] using polarization sensitive OCT.

Discussion

Early caries can be remineralized with therapeutic agents and/or improved oral hygiene, if detected at an incipient stage[22]. However, the effectiveness of these preventive measures can only be determined with a method that can quantitatively monitor the changes in the mineral status of the caries over time[23]. As has been shown[24], there are only a few studies monitoring regression of incipient carious lesions, although it is recognized that incipient carious lesions in smooth surfaces under fluoride therapy can be monitored by optical methods.

A full characterization by OCT of the sound enamel surface is extremely important, because

Table 1. Comparison of layer measurements in mm by the three image systems.

Image system	*n*	Min	Max	Mean	SD	Correlation index (95%)
OCT 840 nm	72	0.130	0.875	0.388	0.174	0.347–0.429
OCT 1300 nm	72	0.137	0.984	0.406	0.188	0.362–0.451
Histology	72	0.130	0.979	0.396	0.175	0.355–0.437

n, number of samples; Min, minimum value; Max, maximum value.
The Student's *t*-test presents *P*-value = 0.823.

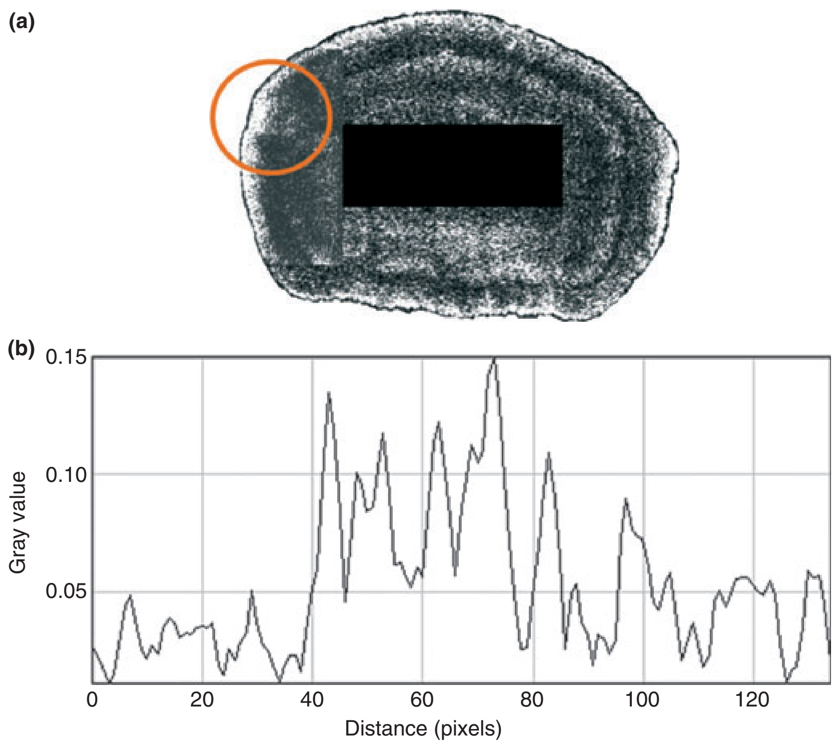

Fig. 5. Evaluation of the contrast on tomographic images (a) caries decay region indicated by the circle; (b) plot of the increase of backscatter intensity in the order of 2–3 times.

it permits that early lesions can be appropriately detected. OCT has already been proven to be a feasible method to detect and determine the dimensions and severity of caries by measuring the loss of penetration depth caused by the increased attenuation due to demineralization[25]. This is particularly important to implement in the proximal surfaces due to uniform optical penetration and surface reflectivity.

Caries evolution in primary teeth is faster than in permanent teeth, and therefore a fast and noninvasive evaluation is imperative. Also, as the enamel thickness is smaller than in permanent teeth, a system with axial resolution of the order of ~10 μm would be advantageous, to promote early detection and quantify the process of remineralization. For clinical devices, it has been shown that SD-OCT has several advantages over TD-OCT, including sensitivity and fast acquisition time, and as the first report on imaging using SD-OCT applied to ophtalmology[26] its use has been widespread.

Conclusions

In accordance with the results presented for the OCT's with wavelengths of 1280 and 840 nm, the two techniques were effective for early caries identification in primary teeth. Furthermore, our experiments demonstrated with the deeper penetration depth of the 1280 nm TD-OCT, and the better clinical advantages of the SD-OCT.

Optical coherence tomography possesses great potential to be used routinely in clinical practice for the complex diagnosis of early enamel caries, promoting possible remineralized preventive measures. Clinical trials by our group for particular dental applications are in progress, which can certainly be further extended to evaluation of primary teeth.

What this paper adds

- This paper adds knowledge about a new technology that is continuously evolving towards being a practical and custom-valued tool for the diagnosis caries.
- The results show that optical techniques applied to early caries detection in primary teeth can be essential for caries diagnosis in children with no pain and no panic, as it is a noninvasive method.

Why this paper is important to paediatric dentist

- This paper is important for paediatric dentists because it discusses about optical properties of enamel structure and caries in primary tooth.
- This paper is also important because in the near future the paediatric dentist will be able to detect and monitor the remineralization of the white spot lesion with the OCT technique.

Acknowledgements

This study was financially supported by the Brazilian agencies CNPq, FACEPE, and CAPES is gratefully acknowledged. The authors also thank the Millennium Institute of Nonlinear Optics, Photonics, and Biophotonics, as part of the MCT/CNPq Millennium Institute program. The Laboratory is part of the Center of Excellence in Nanophotonics and Biophotonics, supported by the PRONEX program FACEPE/CNPq.

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