Comparative analysis of optical coherence tomography signal and microhardness for demineralization evaluation of human tooth enamel

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Abstract

The diagnosis of dental caries at an early stage enables the implementation of conservative treatments based on dental preservation. Several diagnostic methods have been developed, like visual-tactile and radiographic are the most commons but are limited for this application. The Optical Coherence Tomography is a technique that provides information of optical properties of enamel, which may change due to the decay process. The objective of this study was to evaluate the ability of OCT to detect different stages of demineralization of tooth enamel during the development of artificial caries lesions, taking as a reference standard for comparison sectional microhardness testing. Different stages of caries lesions were simulated using the pH cycling model suggested Feathestone and modified by Argenta. The samples were exposed to 0 (control group), 5, 10, 15, 20 and 25 days at a daily regimen of three hours demineralization followed by remineralization during 20 hours. It was used an OCT system with at 930nm. Sectional images were generated in all lesion region. The results obtained from the OCT technique presented similar behavior to microhardness, except for the group 25 days, due to inability to perform indentations reading in areas of more intense demineralization. A linear relationship was observed between the OCT and microhardness techniques for detection of demineralization in enamel. This relationship will allow the use of OCT technique in quantitative assessment of mineral loss and for the evaluation of incipient caries lesions.

Keywords: optical coherence tomography, dental demineralization, caries lesions, optical backscattering

1. Introduction

The diagnosis of carious lesions in early stages of development enables the implementation of conservative treatments, such as fluoride, antibacterial therapies and changes in feed and hygiene habits, avoiding the weakening of the tooth[1]. Several diagnostic techniques have been proposed for detection of incipient caries, since visual-tactile and radiographic methods are limited for this application[2,3]. Many of these techniques, among them Optical Coherence Tomography (OCT), are based in optical principles[4], since the demineralization promotes changes in optical properties of dental enamel[5].

OCT is a non-destructive and non-ionizing technique that provides sectional images with high resolution of light scattering structures, in real time[6]. Its application to biological tissues was first described by Huang *et al.*[7] and had Optical Coherence Domain Reflectometry (OCDR) as precursor[8]. OCT system is based on the Michelson interferometer and a wide bandwidth light source is used, with near-infrared wavelength, because *freitas.az.ipen@gmail.com, phone: +55-11-31339356; www.ipen.br

this region of the spectrum is less absorbed by the main components of biological tissues[9]. The light pass through an optical element that splits the beam into two parts, one part being directed to the mirror of the reference arm and other part is directed to the sample. The light reflected from the mirror present in the

Biophotonics: Photonic Solutions for Better Health Care III, edited by Jürgen Popp, Wolfgang Drexler, Valery V. Tuchin, Dennis L. Matthews, Proc. of SPIE Vol. 8427, 84271H

reference arm and the light backscattered from the sample are recombined at the beamsplitter, where interference between them occurs. The recombinant beam intensity, which is proportional to the square of the electric field resulting, is measured by an optical detector[10].

The diagnostic ability of OCT to analyze simulated caries lesions has been reported in several studies[11,12,13,14,15,16]. The potential of the OCT technique to measure the degree of mineralization of tooth structure is a relevant factor for its use in the study of carious lesions, since it allows assessing the severity and progress of these lesions[13,14,17]. For assessment of the *in vitro* demineralization of tooth enamel, it is important that its surface is maintained (healthy, sound) in order to simulate the initial changes that occur during the development of caries in the oral environment. The pH cycling model described by Featherstone *et al*.(1986) [18] has been used to simulate carious lesions in human teeth for analysis using OCT[14,19]. In order to prevent surface erosion and better simulate the caries lesions, Argenta *et al*. (2003)[20] proposed some modifications for this model[18].

To assess the ability of OCT technique to quantify the degree of mineralization of enamel, it is necessary to compare it to other methods previously established for this assessment, as the sectional microhardness testing, which is a well-recognized method to quantify the mineral changes in enamel and dentin[20,21,22,23], considering that there is a good correlation (0.91) between enamel microhardness and % of mineral in caries lesions[24]. Thus, the purpose of this study was to evaluate the ability of OCT to detect different stages of demineralization of tooth enamel during the development of artificial caries lesions, taking as a reference standard for comparison the cross-sectional microhardness test.

2. Materials and Methods

2.1 Sample preparation and simulation of caries lesions

In this study, 11 sound third molar teeth obtained from the Human Teeth Bank of University of Sao Paulo were used. Their crowns were sectioned in order to obtain 44 slabs, and 2 of which were excluded due to defects in the enamel.

In order to standardize the samples, each block had an area of 6.25 mm^2 delimited by covering the entire sample with an acid resistant varnish (red nail polish – Revlon), except in a window, in which artificial caries lesions was induced. After the artificial lesions were produced, they were performed OCT exams in all samples and then the cross-sectional microhardness testing was conducted.

After preparation, the samples were randomly distributed in 6 groups with 7 samples each (n = 7), according to pH cycling period submitted, as follows: group 0 (control group – no pH-cycling) and groups 1, 2, 3, 4 and 5 were submitted, respectively, to 5, 10, 15, 20 and 25 days of pH cycling regimen.

Artificial caries lesions were produced by pH cycling model simulating the cycle of demineralization and remineralization that takes place naturally in the oral environment[20]. During demineralization, each sample was exposed individually for 3 hours a day in a 40ml aliquot buffer solution containing 2.0 mM calcium, 2.0 mM phosphate, 0.030 ppm fluoride and 75 mM acetate maintained at pH 4.3 and at the temperature of 37° C. After demineralization, each tooth was immersed for 20 hours in a 20 ml remineralization solution composed by 1.5 mM calcium, 0.9 mM phosphate, 150 mMl KCl, 0.050 ppm fluoride and 20 mM cacodylate buffer maintained at pH 7.0 and maintained in 37° C.

2.2 Analysis by OCT

All samples were examined using OCT technique at the beginning of the experiment, and those of groups ranging from 1 to 5 were again subjected to OCT examination after the pH cycling regimen. An OCT system with a superluminescent LED at 930 nm with 2 mW of power was used (Thorlabs Inc.). Images of 4000x1500 microns (2000x512 pixels) were generated in all lesion regions. These images presented axial resolution of 4.0 μm and a lateral resolution of 6.0 μm. The images of the central part of the exposed window were evaluated using software developed in LabView 8 to obtain the total optical attenuation coefficient of

each image. The images obtained from the edges of the caries lesions were excluded from evaluation due to irregularities on the margins caused by acid-resistant varnish application (see Figure 1).

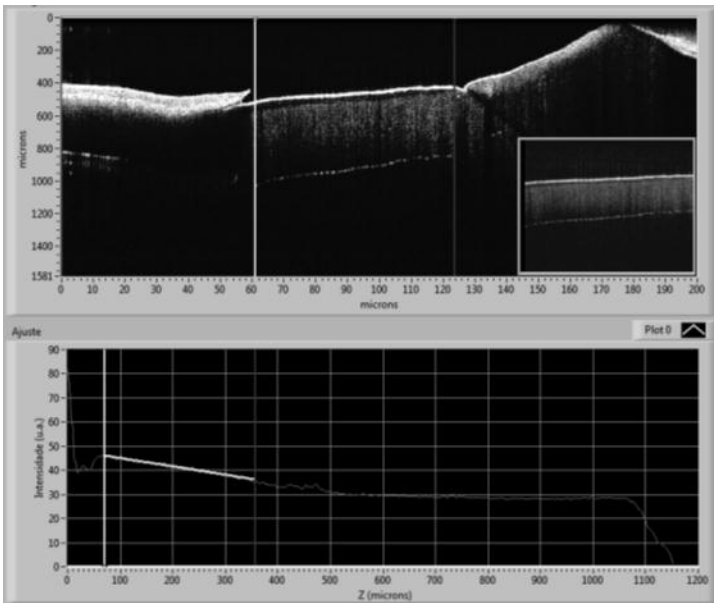

Figure 1. Software developed in Labview to retrieve the attenuation coefficient from OCT image

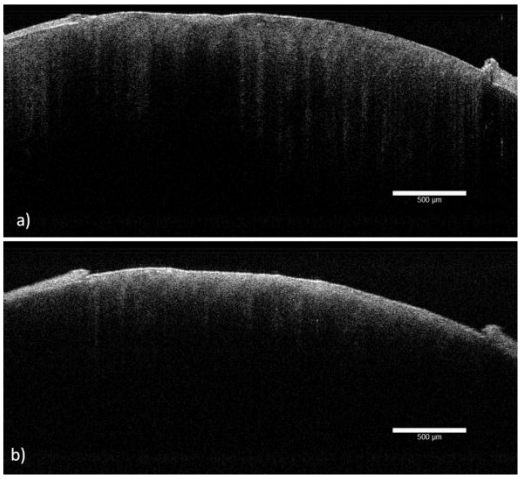

Figure 2. OCT images for health tooth (a) and 15 days of demineralization/remineralization cycling (b).

The total optical attenuation coefficient was calculated from the exponential decay of the detected light intensity (backscattered), according to the equation (1):

$$I(z) = I_0 e^{-2\alpha z} + C \quad (1)$$

where I represents the value of the detected intensity, I_0 is the intensity value of the source in the sample, α is the total optical attenuation coefficient, z is the depth analyzed and C is a constant used due to the background noise signal. The optical attenuation coefficient of each sample was obtained from the arithmetic mean of attenuation coefficient from each of the images evaluated. All data obtained were tested with respect to its normality by the Shapiro-Wilk test. The homogeneity of variances was assessed by Levene's test and,

when necessary, the proper correction was applied to the statistical test. The mean values obtained before and after pH cycling were analyzed using t-test for paired samples, to verify the changes of the each sample as a result of demineralization. The average attenuation coefficients of the groups after pH cycling were also compared to evaluate whether there is statistically significant difference between these measurements for the different periods tested in the experiment.

2.3 Analysis by cross-sectional microhardness

After OCT exams, at the end of the experiment, cross-sectional microhardness testing was performed. The 42 blocks were sectioned to obtain 4 slices of each sample. The slices were embedded in thermally activated acrylic resin (VipCril) and, subsequently, they were polished. The exams were performed using a microhardness tester (2T HMV, Shimadzu). In the central portion of each sample they were performed three columns of indentations, with a distance of approximately 100 μm between them. In each column 15 indentations were made at a distance of 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180 and 200 μm from the edge of the enamel (Figure 3). To perform the indentations it was used load of 25 g applied for 5 seconds, following the model proposed by Argenta *et al*[20].

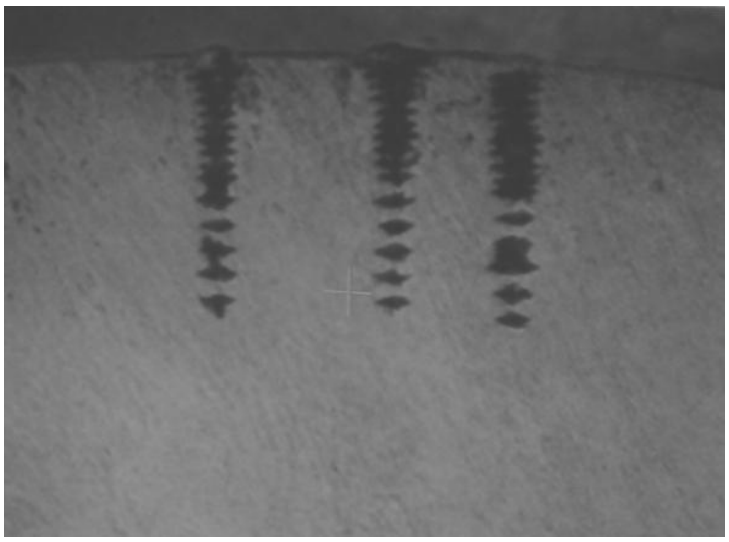

Figure 3. Sample with indentations done with microhardness tester (2T HMV, Shimadzu).

Since hardness is related to the mineral content of the dental element[22], the Knoop hardness number (KHN), provided automatically by the analysis software coupled to microhardness tester, was used to evaluate the mineral content of tooth enamel. For each sample, it was obtained an average value for each depth and for all samples the measurement was performed from edge until 200 μm depth.

3. Results and Discussion

3.1 OCT results

The total optical attenuation coefficient was determined from the OCT signal analysis of the samples, based on the exponential decay of this signal. The values of average attenuation coefficient of each sample were compared before and after demineralization caused by pH cycling, except for group 0 (control), which was examined only once due to not having been subjected to the cariogenic challenge. Comparison between groups was also performed to verify the differences in distinct periods of pH cycling.

The average values of attenuation coefficient for the groups tested are shown in Figure 4. The results were statistically evaluated using paired t-test. To keep the global significance level $\alpha \leq 0.05$, all p-values were corrected by the Ryan-Holm stepdown Bonferroni procedure.

It was observed a statistically significant difference for all groups, in which p values were found as: of 0.00048, 0.0055, 0.011, 0.0064 and 0.019, respectively, for groups 1, 2, 3, 4 and 5. The intensity of the detected signal was higher before enamel demineralization. Similar result was found by Amaechi *et al*.[13] when comparing demineralized and healthy teeth. This happen because the demineralization process creates gaps in structure of the enamel. This way, the number of interfaces increases, and so does the scattering of the light.

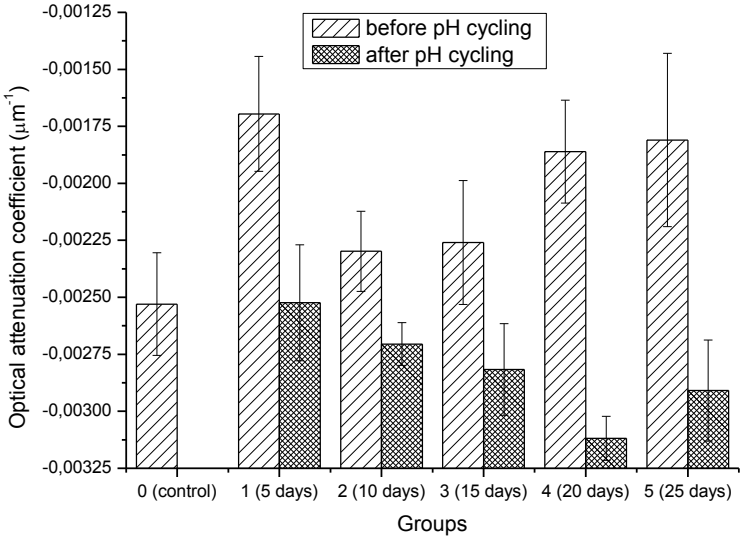

Figure 4. Optical attenuation coefficient for different periods of cycling. The negative signal of the measurements indicates exponential decay of intensity of OCT.

The intergroup comparison was performed using the difference between optical attenuation coefficients obtained before and after pH cycling, and the results were statistically analyzed by ANOVA and Tukey's test (α = 0.05). The results are shown in Figure 5. It is possible to evidence that there is no statistical difference between evaluated groups. These findings can be explained by the natural variability of the composition of the human enamel, which can vary the mineral and organic material from one point to another in the enamel surface of the same tooth. This can reflect on the demineralization process and, in this way, in the optical attenuation coefficient measured.

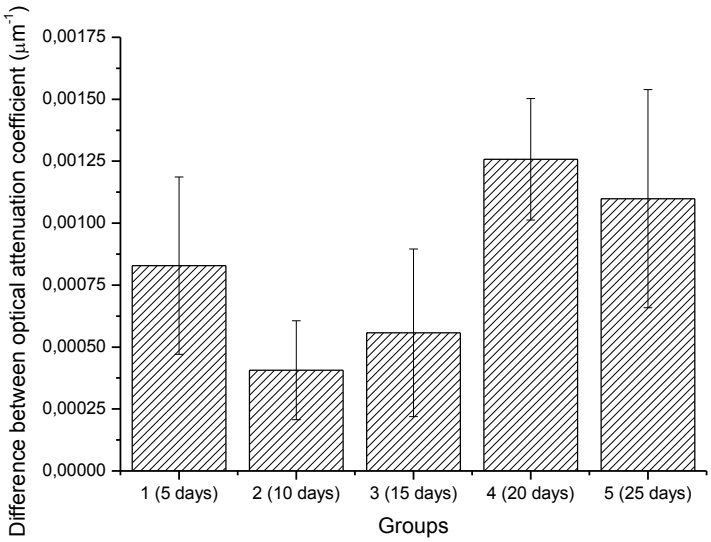

Figure 5. Optical attenuation coefficient difference between control and cycling groups. Difference between optical attenuation coefficients retrieved before and after cariogenic challenge simulation, for each evaluated group.

3.2 Cross-sectional microhardness results

The cross-sectional Knoop microhardness values were plotted as a function of depth for each of the slices of enamel obtained from the section of the samples. They were calculated average microhardness values of all samples for depths evaluated, considering the statistical weight of each measure. It was observed that the microhardness values increased with depth up to approximately 120 μm and from this, suffered little variation in all experimental groups. The average values of microhardness as a function of depth for each group are shown in Figure 6.

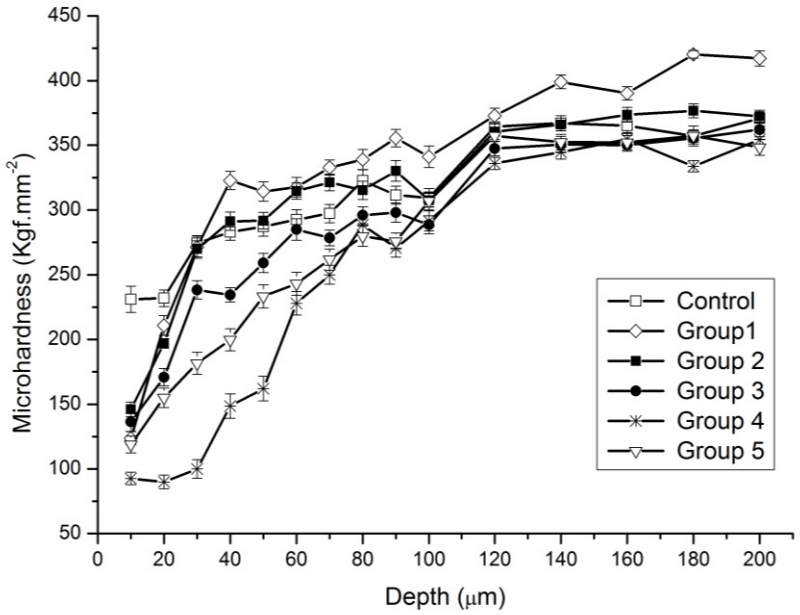

Figure 6. Average microhardness values for each depth. Higher variation is observed until 120 μm depth.

The lowest microhardness values were found for group 4 (20-day pH cycling), suggesting greater demineralization for this group. Although it was expected that group 5 presented the highest demineralization degree due to the higher pH cycling time, some lesions were lost during the cutting of the samples, probably due to the higher degree of demineralization. In this way, the indentations are made in the remaining portion, which has a higher mineral content.

To compare the demineralization levels at different times of pH cycling we considered the microhardness values obtained to a depth of 120 μm, since from this, there was no evidence of loss of hardness. The average values of microhardness for the whole depth considered are presented in Figure 7.

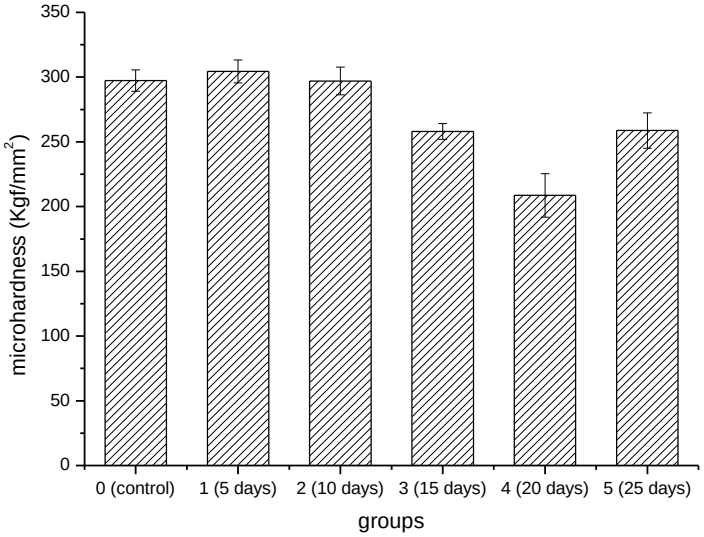

Figure 7. Average microhardness loss values for each group obtained from 10 to 120 μm depth.

Above results were analyzed using analysis of variance (ANOVA) followed by the Tukey post-hoc statistical test, when necessary. Significant difference was observed, with $p < 0.05$, between group 4 (20 days of pH cycling) and Groups 0, 1 and 2. . There was no statistical difference for other comparisons intergroups.

3.3 Comparative analysis: OCT and cross-sectional microhardness results

In order to compare OCT and cross-sectional microhardness techniques, we used the averages of attenuation coefficient and microhardness loss presented by the groups at the end of the period of pH cycling. A linear relation was observed between the normalized results from both compared methods, as shown in Figure 8.

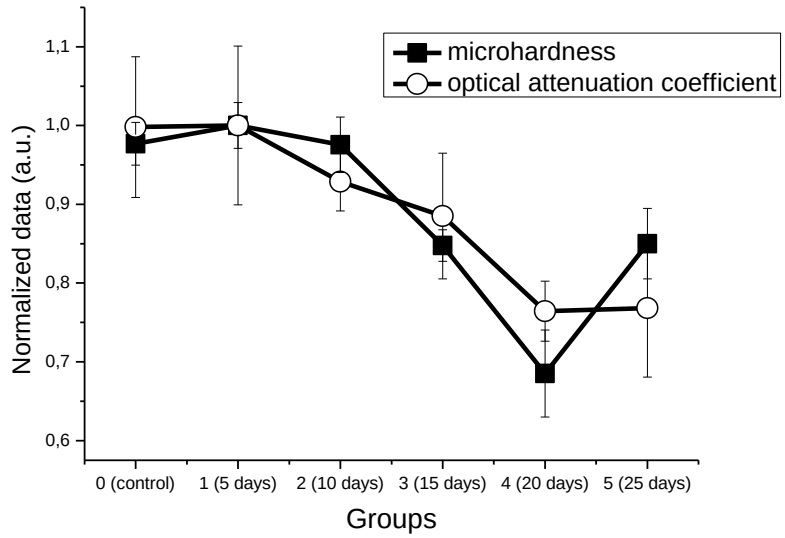

Figure 8. Average results of cross-sectional microhardness obtained until 120 μm depth and optical attenuation coefficient. The results were normalized with respect to the maximum value for both techniques.

The results obtained from the OCT technique presented similar behavior to microhardness, except for group 5, due to inability to perform reading of the indentations in areas of more intense demineralization.

4. Conclusions

In this work it was demonstrated a strong correlation between microhardness measurements of demineralized human dental enamel and the non-invasive, non-destructive technique OCT.

The observed correlation between the total attenuation coefficient determined by OCT scattering signal analysis and the microhardness techniques for detection of demineralization in enamel demonstrates the huge potential for the OCT to detect demineralization of human dental enamel lesions in simulated caries, and even quantitative assessment of mineral loss.

Acknowledgment

The authors would like to thanks FAPESP process #2012/03637-7 and #2010/03123-8 for supporting this research.

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