Characterization of caries progression on dentin after irradiation with Nd:YAG laser by FTIR spectroscopy and fluorescence imaging

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ABSTRACT

Considering the use of high intensity lasers for preventing dental caries, this blind *in vitro* study evaluated the compositional and fluorescence effects promoted by Nd:YAG laser (λ=1064 nm) when applied for prevention of progression of dentin caries, in association or not with topical application of acidulated phosphate fluoride (APF). Sixty bovine root dentin slabs were prepared and demineralized by 32h in order to create early caries lesions. After, the slabs were distributed into six experimental groups: G1- untreated and not submitted to a pH-cycling model; G2- untreated and submitted to a pH-cycling model; G3- acidulated phosphate fluoride application (APF); G4- Nd:YAG irradiation (84.9 J/cm^2, 60 mJ/pulse); G5- treated with Nd:YAG+APF; G6- treated with APF+Nd:YAG. After treatments, the samples of groups G2 to G6 were submitted to a 4-day pH-cycling model in order to simulate the progression of early caries lesions. All samples were characterized by the micro-attenuated total reflection technique of Fourier transformed infrared spectroscopy (μATR-FTIR), using a diamond crystal, and by a fluorescence imaging system (FIS), in which it was used an illuminating system at λ= 405±30 nm. Demineralization promoted reduction in carbonate and phosphate contents, exposing the organic matter; as well, it was observed a significant reduction of fluorescence intensity. Nd:YAG laser promoted additional chemical changes, and increased the fluorescence intensity even with the development of caries lesions. It was concluded that the compositional changes promoted by Nd:YAG, when associated to APF, are responsible for the reduction of demineralization progression observed on root dentin.

Keywords: laser, dentin, FTIR spectroscopy, Fluorescence imaging, caries prevention, fluoride

1. INTRODUCTION

Nowadays it has been noted an increase on the incidence of root caries lesions[1,2], as a result of the higher permanence of teeth in the oral cavity, with consequent gingival recession and root exposure[3]. The increase of root caries lesions is a consequence of the population aging due to the longer life expectancy. When exposed, the root dentin surface is highly susceptible to demineralization than enamel since its mineral content is lower and the hydroxyapatite crystal size is smaller[4]. In this way, it is necessary just a slight decrease in oral pH so that this phenomenon begins and progresses rapidly[5].

Although the use of topical agents based on fluoride is an effective way to prevent the appearance of caries lesions or to avoid caries progression[6], such a measure requires constant repetition taking into account the solubility of the product formed in the saliva[7]. This fact can be a problem for elderly patients or patients with difficulties mobility. Thus, the adoption of measures that act in the exposed substrate is necessary, making it more resistant to the cariogenic challenge.

The dental hard tissue irradiation with high intensity lasers has been considered as a promising therapeutic measure, considering that laser irradiation can promote chemical and crystallographic changes in enamel tissue, making it more resistant to demineralization[8]. The Nd:YAG laser, emitted at wavelength of 1064 nm, has been successfully used to prevent incipient enamel lesions[9]. However, there are no studies in the literature that report the use of this wavelength for preventing root caries progression. In this way, this study aimed to evaluate the compositional and fluorescence changes promoted by the Nd:YAG laser, when associated or not with topical application of the acidulated phosphate fluoride gel, on root dentine demineralization progression.

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2. METHODOLOGY

2.1. Experimental Design

It was performed a blind *in vitro* study in which sixty bovine dentin slabs were prepared and demineralized during 32h in order to obtain simulated early caries lesions. After this period, the samples were randomly distributed into six experimental groups (n = 10): G1- samples untreated and not submitted to a pH-cycling model (only incipient caries); G2- samples untreated and submitted to a pH-cycling model (caries progression – negative control); G3- samples treated with topical application of acidulated phosphate fluoride gel (APF-gel, 1.23% F^-, pH 3.6 to 3.9) during 4 minutes (positive control); G4- samples irradiated with Nd:YAG laser (1064 nm, 60 mJ/pulse, 84.9J/cm^2) after application of a photoabsorber; G5- pre-irradiation with Nd:YAG laser followed by application of APF-gel; G5- application of APF-gel and post-irradiation with Nd:YAG laser. After treatments, the samples of groups G2 to G6 were submitted to a 4-day pH-cycling model in order to simulate the progression of early caries lesions. All samples were characterized by the micro-attenuated total reflection technique of Fourier transformed infrared spectroscopy (μATR-FTIR), using a diamond crystal, and by a fluorescence imaging system (FIS), in which it was used an illuminating system at λ= 405±30 nm. For compositional analysis, it was analyzed the areas under the absorption bands corresponding to phosphate, amide I, amide II, amide III and carbonate contents, and the normalization of spectra was performed by the area of phosphate band. For fluorescence analysis, it was obtained a fluorescence intensity index, in which the fluorescence intensities of samples were normalized by the fluorescence of a sound dentin. The statistical analysis was performed individually for each analysis performed, at 5% significance level. The treatments were considered as a separated block, while the experimental unit was the slab (n = 10).

2.2. Sample preparation

This study was approved by the Animal Ethics Commission of the UFABC (protocol 006/2013). After approval, sixty 8 x 4 x 1 mm of dentin slabs were obtained from the cervical root surfaces of incisor bovine teeth. The slabs were provided from the central area of vestibular root surfaces of all teeth and, after cutting with an air-cooled diamond saw, the prepared slabs were cleaned with fluoride-free pumice and carefully examined under a stereomicroscope in order to verify the absence of irregularities or cracks.

After preparation, all surfaces of specimens were protected with an acid-resistant varnish, except for an area of 8 mm^2 of dentin, where the treatments were applied[10]. All slabs were kept in humid environment under refrigeration during all stages of this study in order to avoid dehydration of samples.

2.3. Creation of an early caries lesion: initial demineralization

The initial demineralization of samples was performed in order to simulate the effects of treatments in an early caries lesion in dentin. For that, all samples were immerged in a demineralizing solution for 32 hours, which induces the formation of a caries lesion with depth of 30 μm[10]. The composition of the demineralizing solution is 1.4 mM calcium, 0.91 mM phosphate, 0.06 μg F^-/mL in 0.05 M acetate buffer, pH 5.0. The blocks were kept at a constant temperature of 37°C and, after this period, all blocks were washed with distilled and deionized water for 15 s and dried with absorbent paper.

2.4. Treatments

The samples were randomized into 6 groups of 10 specimens each (n = 10) and were treated according to Table 1.

In groups G4, G5 and G6, the irradiations were performed using a pulsed Nd:YAG laser device (Pulse Master 1000, ADT, USA), with wavelength of 1064 nm, fixed pulse duration of 150 μs, beam spot size of 300 μm, 10 Hz of repetition rate, mean power of 0.6 W, energy per pulse of 60 mJ and energy density of 84.9 J/cm^2, without air-water coolant, according to previous studies[9]. Before laser irradiations, it was applied a layer of a photoabsorber (coal paste diluted in 50% alcohol) in order to enhance the laser absorption to the surface of dentin[11]. The irradiations were performed by in a scanning way by hand, in order to simulate a clinical condition. Before irradiation and every five irradiated samples, the energy per pulse was calibrated by an energy/power meter (FieldMaster, Coherent, USA).

In groups G3, G5 and G6, it was applied an acidulated phosphate fluoride gel (1.23% F^-, 0.1 M of phosphoric acid, pH 3.6 to 3.9 – Fluor Gel®, Dentsply, Brazil) for 4 minutes with a cotton swab[12]. After this time, the slabs were washed with distilled and deionized water during 1 min and dried with absorbent paper.

Table 1. Experimental groups of the present study.

Group	Denomination	Treatment	pH cycling model
G1	incipient caries	untreated	no
G2	caries progression	untreated	yes
G3	APF application	Application of topical acidulated phosphate fluoride gel (APF)	yes
G4	Laser irradiation	irradiation with Nd:YAG laser	yes
G5	Laser + APF application	irradiation with Nd:YAG laser + application of APF	yes
G6	APF + Laser irradiation	application of APF + irradiation with Nd:YAG laser	yes

2.5. Simulation of the progression of early caries lesions: pH cycling model

The samples of groups G2-G6 were submitted to a 4-day pH-cycling model in order to simulate the progression of the early caries lesions on dentin[10]. For that, the slabs were kept individually in a demineralizing solution for 4 h (6.25 mL/mm^2) and in a remineralizing solution for 20 h (3.12 mL/mm^2) each day, at 37° C. The demineralizing solutions were composed by 1.4 mM calcium, 0.91 mM phosphate, 0.06 ppm F^- in 0.05 M acetate buffer, pH 5.0, and the remineralizing solutions were composed by 1.5 mM calcium, 0.9 mM phosphate, 150 mM of KCl, 0.05 ppm F^- in 0.1 M TRIS buffer, pH 7.0. At the time of changing solutions, all slabs were individually washed with distilled and deionized water for 15 s and dried with absorbent paper and, then, returned to the same solution of the previous day.

2.6. Compositional analysis

The compositional analysis was performed on all samples; for each sample of each experimental group three spectra were obtained: one was obtained on sound samples, other was obtained after initial demineralization and a third one was obtained immediatelly after treatments. For that, it was used the attenuated total reflectance technique of the Fourier transform infrared micro-spectroscopy (μATR-FTIR). The ATR-FTIR spectra of each sample were obtained with 4.0 cm^{-1} resolution, on a Varian 610 spectrometer equipment (Varian Inc., EUA), with a germanium crystal. Each spectrum had a background spectra subtracted during acquisition and was obtained with 80 scans in the range of 4000 to 600 cm^{-1}.

After selection of the bands of interest, the background signal was subtracted and, for a semi-quantitative comparison between groups, the areas under the considered bands were calculated after normalization by the area of phosphate band (1300–900 cm^{-1})[13]. The absorption bands considered for this study were the ν_2 vibration mode of carbonate (around 870 cm^{-1}), the superposition of the stretching ν_3 and bending ν_4 vibration mode of carbonate (between 1600-1300 cm^{-1}), amide I (1680-1600 cm^{-1}), amide II (1580-1480 cm^{-1}) and amide III (1200-1300 cm^{-1})[14].

All the obtained spectra were recorded in the binary format and converted into ASCII format for data analysis, using the Varian Resolutions Pro Software (Varian Inc., EUA). After that, normalization of all spectra and the calculation of area under the infrared bands were performed using the Origin 8.0 Software.

2.7. Fluorescence analysis

For all treatment groups, the changes in fluorescence of the samples were determined in three stages: after the initial demineralization, after treatment and after the pH cycling. For this purpose, it was used a fluorescence imaging system (FIS) based on a mechanical wheel broadband optical filters (λ= 450nm, Finger Lakers Instrumentation, USA), a scientific CCD camera (Matrix Vision, Germany), an objective lens (Edmund Optics, USA) and an illumination system composed by LEDs (λ = 405 ± 30 nm), controlled by computer[15]. For all measurements, a sound and hydrated dentin sample was kept as a reference, and it was positioned laterally to the analyzed sample, as it can be shown in Figure 1. The power of the LEDs was monitored during all the experiments using a powermeter (FieldMaxII, Coherent, USA).

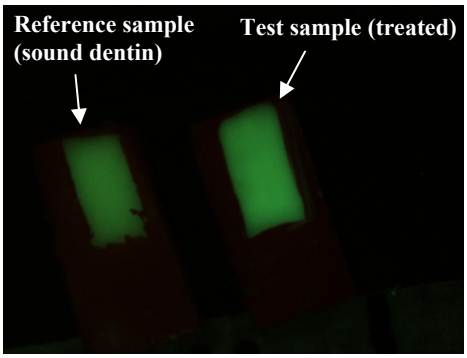

Figure 1. Fluorescence image obtained during the experiments, evidencing the positioning of samples during the analysis.

After the acquisition of the images, the analysis of the fluorescence intensity of each sample was obtained considering the G component of the RGB system, using a Matlab (Mathworks, USA) routine. For this analysis, a standardized area that corresponded to all exposed dentin was considered. The value of the fluorescence intensity of each sample was calculated using an index, obtained by dividing the value of fluorescence intensity of the test sample by the value of the reference one.

2.8. Data treatment

For comparison of compositional changes of sound and demineralized dentin, it was used the *t*-Student test. For the analysis considering all the experimental groups, the differences among treatments were analyzed by Kruskal-Wallis followed by Student-Newman-Keuls test. For all analyses, 5% was considered the limit of significance and the software SPSS 13.0 for windows (SPSS Inc., Chicago, IL) was used.

3. RESULTS

The Figure 2 shows the normalized μ-ATR/FTIR spectra of the root dentin samples before (sound dentin) and after the initial demineralization (incipient caries). These spectra display all the peaks corresponding to the mineral content of dentin tissue — 826-888 cm^{-1} (ν_2 carbonate vibration), 888-1185 cm^{-1} (ν_3 phosphate vibration), 1300-1516 cm^{-1} (ν_3 and ν_4 carbonate vibration); as well as the peaks corresponding to the organic content —1590-1720 cm^{-1} (amide III vibration), 1515-1590 cm^{-1} (amide II + carbonate vibration) and 1185-1300 cm^{-1} (amide III vibration). The vibrations of water (2500-3664 cm^{-1}) and of the C-H stretching mode of the lipids (2835-2980 cm^{-1}) were showed in this spectrum but they were not considered for statistical analysis.

After demineralization, it can be noted that no new bands or the disappearance of bands were evident. However, the intensities of peaks associated with carbonate, phosphate and water contents are decreased after demineralization. On the contrary, the content of amides I, II and III seems to be unaffected after the demineralization process.

The Figure 3 shows the normalized absorption area of all organic and carbonate content of root dentin before and after demineralization. It is confirmed a statistically significant ($p < 0.05$) decrease on the ratio of ν_2, ν_3 and ν_4 vibrations of carbonate/phosphate after demineralization. On the other hand, it was evidenced the significant increase on the organic content (amide II and amide III) of dentin in relation to phosphate after demineralization ($p < 0.05$).

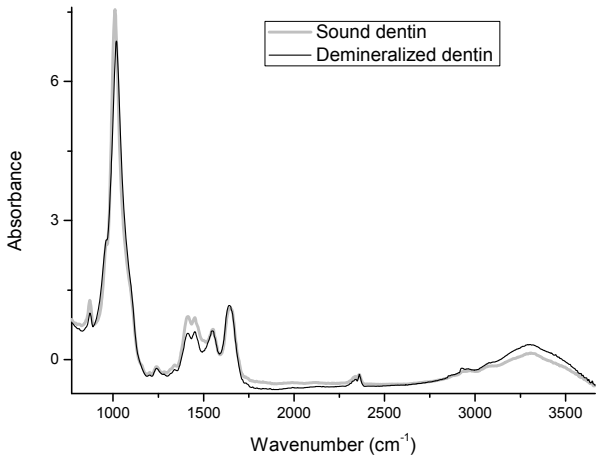

Figure 2. Infrared absorption spectrum of bovine root dentin before and after demineralization. It is possible to evidence a decrease in carbonate and phosphate peaks, as well as an increase on the content of water.

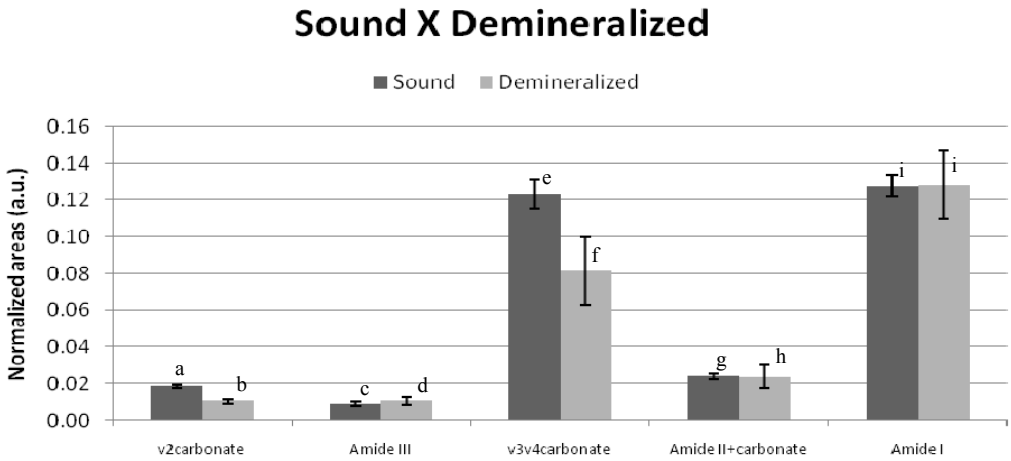

Figure 3. Mean and standard deviation (bars) of the normalized areas under the absorption bands of carbonate and amides before and after initial demineralization. Distinct letters indicate statistically significant differences according to *t*-Student test.

The μATR-FTIR averaged spectra of all treatment groups is shown in Figure 4, where it can be observed changes on inorganic, organic and water contents of dentin, depending on the treatment applied. None of treatments

promoted the formation or disappearance of infrared bands. The Table 2 shows the semi-quantitative analysis of the infrared bands after normalization by the phosphate band, and it is possible to evidence an increase ($p < 0.05$) on the organic content of dentin (amides I and II) after APF application when compared to incipient caries group (G1); however, the APF application did not alter the carbonate/phosphate or amide III+carbonate/phosphate ratio when compared to G1. Laser irradiation alone did not promote any additional changes on both inorganic or organic content of dentin when compared to G1 group; on the other hand, the association of laser irradiation with APF application promoted an increase on the ratio of amides I/phosphate and amide II/phosphate mainly when APF was applied after laser irradiation. When irradiations were performed after APF application, it was only noted a significant increase on amide I content when compared to G1 group.

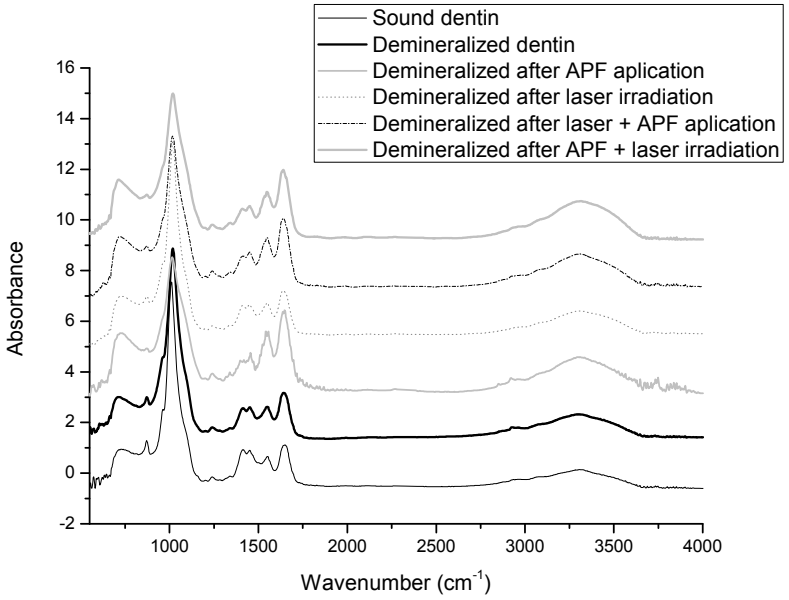

Figure 4. Average μATR/FTIR spectra of the samples of demineralized bovine root dentin after the application of the proposed treatments. All spectra had vectorial normalization.

Table 2. Mean ± standard deviation (arbitrary units) of areas under the infrared absorption bands, after normalization by phosphate band, of all experimental groups, immediately after treatments*.

Groups	Carbonate	Amide I	Amide 2	Amide 3
Sound dentin	0.013 ± 0.001^{a}	0.105 ± 0.008^{a}	0.017 ± 0.003^{a}	0.007 ± 0.001^{a}
G1 (incipient caries)	$0.010 \pm 0.001^{b.c}$	0.128 ± 0.018^{b}	0.023 ± 0.006^{b}	$0.011 \pm 0.001^{b.c}$
G2 (caries progression)	$0.010 \pm 0.001^{b.c}$	0.128 ± 0.018^{b}	0.023 ± 0.006^{b}	$0.011 \pm 0.001^{b.c}$
G3 (APF application)	0.004 ± 0.002^{b}	0.283 ± 0.166^{c}	0.067 ± 0.045^{c}	$0.023 \pm 0.001^{b.c}$
G4 (laser irradiation)	0.010 ± 0.001^{c}	0.128 ± 0.017^{b}	0.025 ± 0.007^{b}	0.010 ± 0.003^{b}
G5 (laser + APF)	$0.007 \pm 0.004^{b.c}$	0.247 ± 0.137^{c}	0.049 ± 0.029^{c}	0.023 ± 0.017^{c}
G5 (APF + laser)	$0.005 \pm 0.001^{b.c}$	0.219 ± 0.137^{c}	$0.037 \pm 0.016^{b.c}$	$0.012 \pm 0.010^{b.c}$

*Distinct letters indicate significant differences according to Student-Newman-Keuls test. The comparison was performed among rows.

The analysis of the fluorescence intensity index showed that the initial demineralization process caused a significant loss of fluorescence intensity when compared to the fluorescence of the sound dentin (G1 group, Table 3). After treatments, the application of APF promoted further decrease on fluorescence intensity; however, laser irradiation, in combination or not with APF, did not cause additional changes in the fluorescence of the samples.

After the pH cycling, it was observed that both the topical application of APF as laser irradiation did not cause significant changes in the fluorescence intensity of the samples in relation to the demineralized dentin group (G2); however, the G5 group (laser irradiation followed by APF application) promoted a significant increase in the fluorescence intensity, whose values were similar to the sound dentin (G1 group).

Table 3. Mean ± standard deviation (arbitrary units) of fluorescence intensity index (arbitrary units) of all experimental groups of the present study*.

Groups	After treatments	After pH cycling
Sound dentin	0.98 ± 0.0^{a}	-
G1 (untreated, incipient caries)	0.76 ± 0.1^{b}	-
G2 (untreated, caries progression)	0.76 ± 0.1^{b}	0.49 ± 0.2^{c}
G3 (APF application)	0.54 ± 0.2^{c}	0.54 ± 0.2^{c}
G4 (laser irradiation)	0.76 ± 0.3^{b}	0.61 ± 0.2^{c}
G5 (laser + APF)	0.80 ± 0.2^{b}	0.81 ± 0.2^{b}
G5 (APF + laser)	$0.90 \pm 0.3^{a,b}$	0.76 ± 0.2^{b}

*Distinct letters indicate significant differences according to Student-Newman-Keuls test.

4. DISCUSSION

Although the use of high intensity laser irradiation is widely reported for preventing caries appearance and progression on enamel, there are few studies concerning the possibility of using the Nd:YAG laser for preventing dentin caries. As well, there are no studies that report the possibility of using this laser system for preventing root caries progression.

In this work, the simulation of the root caries progression was performed by a validated *in vitro* pH-cycling model, which aimed to standardize and to control the variables found on the natural caries. The literature reports that the initial demineralization process performed in this study, by the time of 32 h, induces measurable caries-like subsurface lesions without surface erosion[10], allowing the evaluation of mineral loss by several techniques, including microhardness and FTIR spectroscopy.

When analyzing the infrared spectra of root dentin specimens with this initial demineralization (incipient caries lesions), it was evidenced the decrease of phosphate and carbonate content when compared to sound root dentin, which indicates the occurrence of mineral loss at the surface of the samples. It is important to emphasize that the analysis by μATR-FTIR spectroscopy provides chemical measurements up to 6 μm in depth (previously calculated considering as 1.63 the refraction index of teeth). It was also observed the significant decrease on the ratio of ν_2, ν_3 and ν_4 vibrations of carbonate/phosphate after demineralization, which suggests that the carbonate was partially lost during the demineralization process, and this loss was higher than the phosphate component. The carbonate content of dental hard tissues is known to vary depending on the stage of maturation[16]. In this way, periods of demineralization and remineralization results in a replacement of the carbonate ions and, considering that it was induced an initial caries lesion on root dentin, it is expected that the carbonate is lost during the demineralization process. The increase on the organic content (amide II and amide III) of dentin in relation to phosphate after demineralization evidences the occurrence of the loss of phosphate and the exposition of organic matrix during demineralization, which is also expected during the carious process.

The application of APF on demineralized dentin promoted the increase on the proportion of amide I/phosphate and amide II/phosphate. This fact can be due to the action of phosphoric acid present on APF gel, which partially dissolves the surface in order to form a higher amount of CaF_2-like material[7], and can even expose a higher amount of organic matrix in this process. In this work, it was observed that the Nd:YAG laser alone does not promote additional compositional changes in the demineralized dentin structure, which contradicts the published findings that report that laser irradiation increases enamel crystallinity and reduces the carbonate content and the organic matrix of enamel[17]. However, it is important to emphasize that dentin has lower mineral content and higher organic matrix when compared to enamel and, in this way, the effects of an infrared laser irradiation can be different. As well, in the present study, the root dentin presented an even less content of phosphate and carbonate due to the incipient caries lesion induced. In this way, the effects of laser irradiation on this specimen were lesser than the reported for enamel.

The analysis by FIS showed a decrease in fluorescence index of incipient caries lesions when compared to sound dentin, which corroborates the literature studies performed in enamel, which reported that initial carious lesions present less fluorescence intensity than healthy enamel[18]. The changes in the green fluorescence should be considered as the indicator of caries[19], since the loss of fluorescence has been shown to correlate with the higher degree of demineralization[20]. In this study, the results of fluorescence analysis are confirmed by FTIR spectroscopy, in which it was showed the loss of phosphate and carbonate in caries-like subsurface lesions, even without surface erosion[10]. In this way, it can be considered that both FTIR and FIS systems are suitable for diagnosis of incipient dentin caries. When comparing the fluorescence index of incipient caries (G1) and caries progression (G2) groups, it can be noted a significant decrease on this index, suggesting that FIS technique is able to detect root dentin caries progression, showing a high sensitivity and specificity, which agrees with other commercially available systems based on fluorescence[18,19,20,21].

Concerning the treatments of the present study, it was evidenced that the APF-gel application decreased the fluorescence of carious dentin, while Nd:YAG laser irradiation did not promote changes on this aspect. The decrease on fluorescence due to APF-gel application can be due to the remaining of the constituents of the gel on the surface of dentin, or even due to the large formation of CaF_2-like material on the surface[6]. However, further studies are necessary to confirm these hypotheses. Since the Nd:YAG laser did not promote significant compositional changes on root carious dentin, the result of fluorescence obtained for G4 group is expected.

After the pH-cycling, it was noted that the fluorescence index of specimens treated with APF-gel did not change, which suggest that the caries lesion progression was inhibited. Considering that the pH-cycling model used in this study had a short duration (4 days) and that the demineralization and remineralization solutions were not changed during the experiments, the higher amount of CaF_2-like material formed due to APF-gel application was able to prevent the progression of dentin caries. Further microhardness and microscopy analysis are necessary to confirm the absence of changes on mineral loss and on lesion depth after the pH-cycling period.

Nd:YAG laser irradiation alone decreased the fluorescence index of specimens after pH-cycling; however, this reduction was lesser than that observed on untreated samples. In this way, it can be supposed that laser irradiation decreased the caries progression, but laser treatment was not able to inhibit this process. In fact, it has been reported that high intensity infrared laser irradiation can reduce the carbonate content and leads to the evaporation of water, the decomposition of the organic matrix, as well it is related the oxidation of phosphates and the conversion of phosphates to pyrophosphates[22,23]. These actions are mainly due to the temperature increases during irradiations, and it is related with the composition of the dental hard tissues. Dentin has less mineral and higher water and organic content than enamel and, in this way, there are some differences in thermal properties of these tissues[24]. As a consequence, the effects of laser irradiation on reducing the caries progression on a previously demineralized dentin (with lesser content of mineral and carbonate than sound dentin) can be lesser than the effects observed on sound enamel. In the present study, laser irradiation can have induced some structural changes on demineralized dentin that were not detected by FTIR spectroscopy, such as morphological or crystalline changes, and they were enough to reduce caries progression when compared to negative control group; however, the effect of APF-gel was better than laser irradiation alone.

On the other hand, the association of laser irradiation and APF-gel application did not promote changes on the fluorescence index of specimens after pH-cycling, which indicates that the association of treatments can inhibit caries progression. In this way, the most promissory treatment seems to be laser irradiation before APF-gel application (G5 group), which promoted values of fluorescence index similar than those obtained on the incipient caries group (G1). Previous studies relate that laser irradiation increases the formation and the retention of CaF_2-like material on enamel formed due to the topical application of APF-gel[25,26]; in this way, due to the structural changes promoted on irradiated dental hard tissues, laser irradiation can increase the fluoride ion available during the cariogenic process, which

contributed to the prevention of caries progression observed in this study. Once more, further studies are necessary in order to verify the amount of CaF_2-like material formed and retained after Nd:YAG laser irradiation on carious dentin, and also to determine the relationship with the morphological and crystalline changes promoted by irradiations on this tissue. According to the results obtained in this present study, the association of Nd:YAG laser followed by APF-gel application seems to be a promissory and well-effective treatment for inhibiting the progression of incipient root caries lesions.

5. CONCLUSIONS

It was possible to conclude that the reduction of phosphate and carbonate contents on dentin due to the demineralization process are responsible for the decrease on fluorescence intensity of this tissue, when excited whith LEDs that emit on 405 nm. As well, the Nd:YAG laser, when associated to APF application, promote changes mainly on the organic content of root dentin, and these compositional changes can be an important reason for the reduction of root caries progression observed by fluorescence analysis.

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