Advances in the prevention and monitoring of root dentin demineralization using lasers

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Abstract—**The increase in the life expectancy and the longer permanence of the teeth in the oral cavity also led to an augment in the prevalence of root caries lesions. These lesions require more attention because of their rapid progression and difficulty in early diagnosis and monitoring. In this context, the irradiation of the tissues with high intensity lasers has been shown as an important way for preventing lesion formation because lasers chemically modify the irradiated dental hard tissues and make them more resistant to acid challenge. In addition, the association with ceramic biomaterials may allow additional remineralizing results. Together with therapeutics, the effective early diagnosis of incipient lesions is indispensable. Techniques that use lasers, such as optical coherence tomography, have also been promising in this aspect because they allow the early diagnosis and monitoring of demineralizations with high resolution and in a non-detrimental way. This article aims to show the actions of high intensity laser therapies when associated or not with biomaterials, on the prevention and remineralization of root caries lesions, as well as the effectiveness of optical coherence tomography in the diagnosis and monitoring the effects of the treatments in these lesions.**

Keywords—laser, caries, dentin, optical coherence tomography, biomaterial

I. INTRODUCTION

The increase in the prevalence of root caries lesions is a consequence of the higher permanence of the teeth in the oral cavity. This fact can be attributed to the longer life expectancy of the people and improving oral health care. With this, preventive strategies become more necessary, seeking to protect the exposed root surfaces. Dentin is a tissue highly susceptible to demineralization, requiring little pH changes for a carious lesion to begin [1]. Thus, long-lasting methods that make this tissue more resistant are strictly necessary.

In this context, the irradiation with high intensity lasers is a good alternative, considering the chemical changes promoted due to the local temperature increase during the irradiation [2,3]. The association of laser irradiation with bioactive materials is also a possible strategy, as these materials can help to remineralize an incipient lesion [4].

In addition to preventive strategies, the noninvasive monitoring of lesion reversal is strictly necessary and, for this reason, the technique of optical coherence tomography (OCT) is an effective tool because it allows the quantification and qualification of the remineralization processes [5]. In this work, therefore, we aimed to evaluate the effects of Nd:YAG and Er,Cr:YSGG lasers in the prevention of caries and erosion lesions, and also in the remineralization of these lesions, when used alone or associated with a bioactive glass ceramic. It was also evaluated the potential of the OCT in the follow-up of these processes.

II. MATERIALS AND METHODS

A. Experimental design

This *in vitro* study was splitted into 4 experimental phases in which they were used bovine root dentin slabs as samples. In the first, the effects of Nd:YAG laser (1064 nm) when associated or not with topical application of fluoride gel (APF-gel) were evaluated on root erosion. In the second experimental phase, the effects of Er,Cr:YSGG laser (2078 nm) also when associated of not with APF-gel were evaluated on root caries. In the third, it was verified the effects of Er,Cr:YSGG laser associated or not to a bioactive glass-ceramic on dentin remineralization and, at the end, it was observed the morphological changes on root dentin after *Q-switched* Nd:YAG laser irradiation after the bioactive glass-ceramic treatment. The morphological effects were observed using scanning electron microscopy (SEM), as well as the effects of treatments on erosion, caries and remineralization were quantified using optical coherence tomography (OCT). The statistical analysis was performed considering the level of significance of 5%.

B. Nd:YAG laser on erosion

68 bovine root dentin slabs were prepared and randomly distributed into four experimental groups (n = 17) for treatments: untreated (negative control), treated with APF-gel (positive control, acidulated phosphate fluoride, Flutop Gel®, SSWhite, RJ, Brazil, 1.23% F^-, pH = 3.6 to 3.9, 4 min), Nd:YAG laser irradiation; and APF-gel followed by Nd:YAG laser irradiation.

For irradiations, it was used a Nd:YAG laser (Power LaserTM ST6, Lares Research, USA), λ = 1.064 nm, 100 µs, 10 Hz, 0.6 W, 60 mJ, 84.9 J/cm^2. The irradiations were

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performed manually, after the application of a coal paste as a photoabsorber, as previously described [3].

The morphological evaluation was done by scanning electron microscopy (JEOL JSM-6010LA, Tokyo, Japan) immediately after treatments on 2 samples of each experimental group, while the other 15 samples of each experimental group were submitted to an erosive/abrasive challenge during 5 days [6]. OCT evaluations were made after treatments and after erosive/abrasive challenge using an OCT (OCP930SR, Thorlabs Inc., NJ, USA), with 2 mW of optical power, central wavelength of 930 nm, spectral bandwidth of 100 nm, axial resolution of 4.0 μm and lateral resolution of 6.0 μm.

For evaluation of obtained images, it was calculated the optical attenuation coefficient (μ) according to the described by Cara *et al.* (2014) [7]. It was performed the statistical analysis using ANOVA + Tukey´s test, at 5% significance level.

C. *Er,Cr:YSGG laser on early caries*

After preparation, 68 bovine root slabs were distributed into four experimental groups (n = 17): untreated; treated with APF-gel, Er,Cr:YSGG laser irradiation; and APF-gel followed by Er,Cr:YSGG laser irradiation.

For irradiations, it was used an Er,Cr:YSGG laser (Waterlase MD, Biolase Inc. USA), λ = 2.078 nm, 20 Hz, 6 J/cm^2, 8,67 mJ, without air/water cooling. It was used a high-precision motorized translator (ESP300, Newport Corporation, USA) adjusted at 7.6 mm/s for the irradiations, as previously described [8].

SEM evaluations (Quanta, FEI, USA) were made immediately after treatments on 2 samples of each experimental group, while the other 15 samples of each experimental group were submitted to an cariogenic challenge during 8 days, according to Queiroz *et al.* (2008) [9]. OCT evaluations were made after treatments and after cariogenic challenge using an OCT (Callisto, Thorlabs Inc., USA) with central wavelength of 930 nm, pixel resolution of 1.52 μm x 3.34 μm, lateral resolution of 8 μm and axial resolution of 7 μm. It was calculated the optical attenuation coefficient using the methodology proposed by Maia *et al.* (2016) [10]. The comparison among groups was done by ANOVA + Tukey´s test, at 5% significance level.

D. *Er,Cr:YSGG laser and bioactive glass-ceramic on early caries*

85 bovine root dentin slabs were demineralized in order to simulate an early caries lesion according to Queiroz *et al.* (2008) [9]. After, they were randomly distributed in five experimental groups (n = 17): untreated; treated with APF-gel, treated with a bioactive glass-ceramic (Biosilicato®); treated with Er,Cr:YSGG laser irradiation; and bioactive viitroceramic followed by Er,Cr:YSGG laser irradiation.

After treatments, the caries remineralization was simulated during 8 days using a pH-cycling model [9]. The SEM evaluations were performed on 2 samples of each experimental group immediately after treatments, while OCT examinations and the calculation of optical attenuation coefficient were achieved on 15 samples of each experimental group after pH-cycling according to the described before. The statistical analysis was done using Kruskal-Wallis and Student-Newman-Keuls (α = 5%).

E. *Q-switched Nd:YAG laser and bioactive glass-ceramic on dentin morphology*

8 bovine root dentin slabs were prepared and randomly distributed into four experimental groups (n = 2) for treatments: Nd:YAG laser irradiation without the photoabsorber (coal paste [3]); bioactive glass-ceramic application (Biosilicato®); Nd:YAG laser after coal paste application; and bioactive glass-ceramic + Nd:YAG laser after coal paste application.

For irradiations, it was used a Nd:YAG laser (Quantel, USA), 1.064 nm, 5 ns, 0,44 J/cm^2. The irradiations were made manually. SEM evaluations were done immediately after treatments (Quanta, FEI, USA).

III. Results and Discussion

A. *Nd:YAG laser on erosion*

Fig. 1 shows the morphology of sound root dentin untreated (Fig. 1A) and after treatment with APF-gel (Fig. 1B), Nd:YAG laser (Fig. 1C) and APF-gel followed by Nd:YAG laser. It is noticed an opening of dentin tubules supported by APF-gel because of the phosphoric acid constituting this gel. Nd:YAG laser obliterated the dentin tubules and stimulated the formation of a rough lava-like surface, characteristic of melting and recrystallization of dentin and similar to the reported on literature [11]. After APF-gel followed by laser irradiation, it was observed a smoother surface with opening of the dentinal tubules (Fig. 1D). This effect is due to the interaction of the laser with the APF-gel, whose heating caused the evaporation of the smear layer, but not the melting of dentin surface because the photons were retained in the gel, which restricted the propagation of heat.

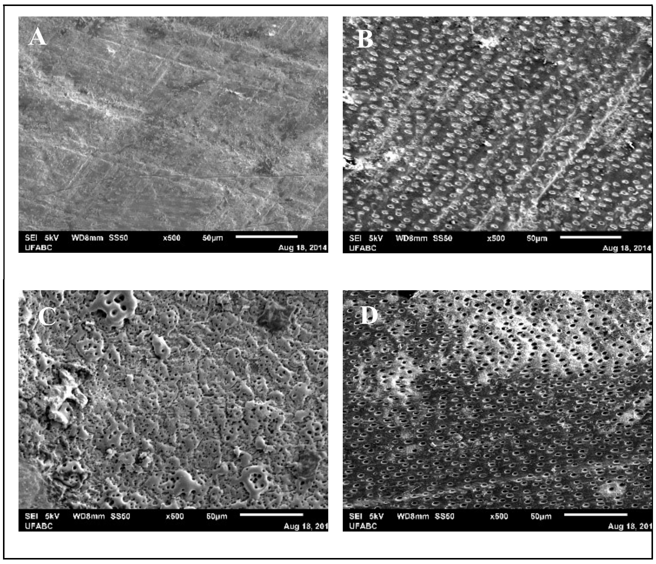

Fig. 1. Eletromicrographs of root dentin untreated (A), after treatment with APF-gel (B), Nd:YAG laser (C) and APF-gel + Nd:YAG laser (D). Original magnification = 500 x.

Representative images from the same samples as observed by OCT are illustrated in Fig. 2. The APF-gel application did not result in significant changes on OCT images (Fig. 2B), but Nd:YAG laser irradiation associated or not with APF-gel increased the backscattering of samples (Fig. 2C and D).

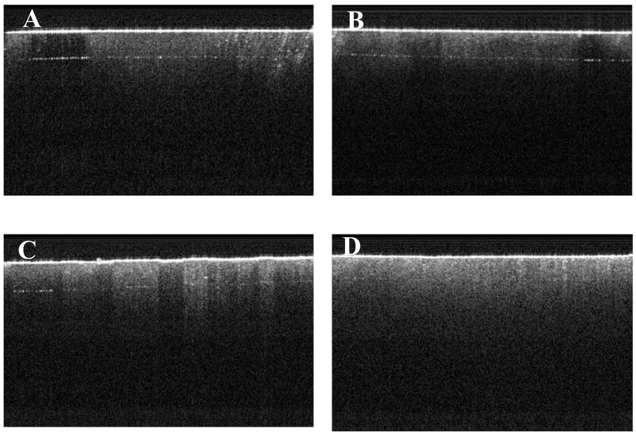

Fig. 2. Representative OCT images obtained from root dentin after treatments: (A) untreated; (B) treated with APF-gel; (C) treated with Nd:YAG laser; (D) treated with APF-gel+Nd:YAG laser.

This indicates that the thermal effects of laser irradiation modify the passage of light through the dentin. The effects promoted by laser irradiation include loss of carbonate and evaporation of water and organic material, as well as formation of new crystalline phases and crystals of increased size [12]. With this, there can be the formation of spaces that increase the backscatter of light.

The analysis of the difference on optical attenuation coefficients (Δμ) after 5 days of erosive challenge (Fig. 3) reveals that Nd:YAG laser, when associated or not with APF-gel, significantly decreased ($p < 0.05$) the Δμ when compared to the groups untreated or treated with APF-gel alone. This finding suggests that Nd:YAG laser reduced the erosion progression on dentin [12], probably due to its thermal effects, in the same manner as reported in enamel studies [13, 14].

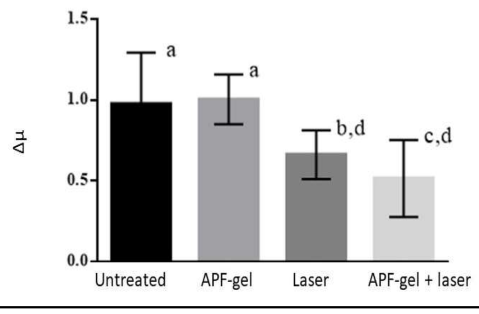

Fig. 3. Means of the difference on optical attenuation coefficients (Δμ) after 5 days of erosive challenge of root dentin untreated, treated with APF-gel, Nd:YAG laser and APF-gel + Nd:YAG laser. Bars denote standard deviation. Distinct supercript letters indicante statistical differences according to the Tukey´s test ($p < 0.05$).

B. Er,Cr:YSGG laser on early caries

Fig. 4 illustrates the morphological changes of root dentin after treatment with Er,Cr:YSGG laser associated or not with APF-gel. It is noticed dentin tubules partially closed after APF-gel treatment (Fig. 4B), whereas Er,Cr:YSGG laser irradiation promoted the formation of irregular surface, with typical ablation morphology (Fig. 4C), which agrees with the literature [15]. This pattern repeats even when the laser was applied after APF-gel treatment (Fig. 4D).

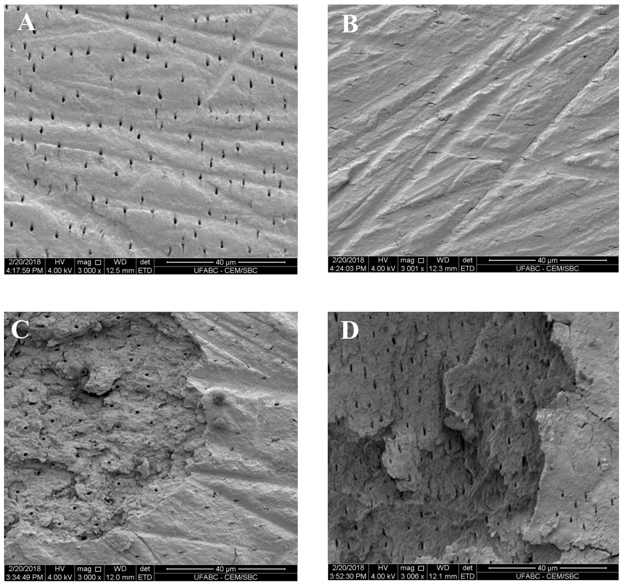

Fig. 4. Eletromicrographs of root dentin untreated (A), after treatment with APF-gel (B), Er,Cr:YSGG laser (C) and APF-gel + Er,Cr:YSGG laser (D). Original magnification = 3000 x.

The OCT images show that Er,Cr:YSGG laser irradiation promotes the formation of brighter areas, mainly located in the regions of the laser pulses (Fig. 5).

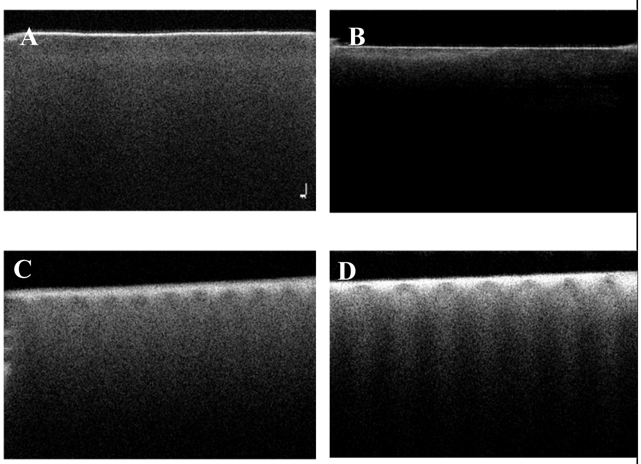

Fig. 5. Representative OCT images obtained from root dentin after treatments: (A) untreated; (B) treated with APF-gel; (C) treated with Er,Cr:YSGG laser; (D) treated with APF-gel+Er,Cr:YSGG laser.

This fact agrees with what was observed with the Nd:YAG laser, that is, regions of greater changes due to heat are those that present a greater backscatter of light. Previous studies performed by our group [16, 17] show that the Er,Cr:YSGG laser, even at low energy densities, promotes compositional and crystallographic changes in dental hard tissues, which may interfere with the passage of light during OCT imaging.

The calculation of optical attenuation coefficient revealed that Er,Cr:YSGG significantly decreased this value when compared to the untreated samples, even associated or not with APF-gel treatment (Fig. 6). This fact suggests that the thermal effect reduced dentin demineralization [7], but the association of treatments did not promote any additional effect.

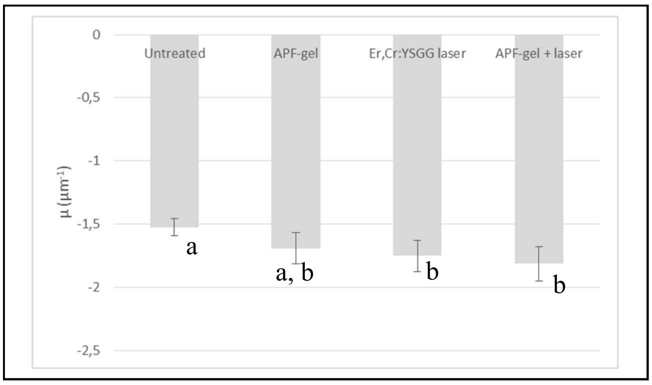

Fig. 6. Means of the optical attenuation coefficients (μ) after 8 days of carious challenge of root dentin untreated, treated with APF-gel, Er,Cr:YSGG laser and APF-gel + Er,Cr:YSGG laser. Bars denote standard deviation. Distinct supercript letters indicante statistical differences according to the Tukey´s test ($p < 0.05$).

C. *Er,Cr:YSGG laser and bioactive glass-ceramic on early caries*

Fig. 7 illustrates the morphological changes of carious root dentin after pH-cycling for 8 days.

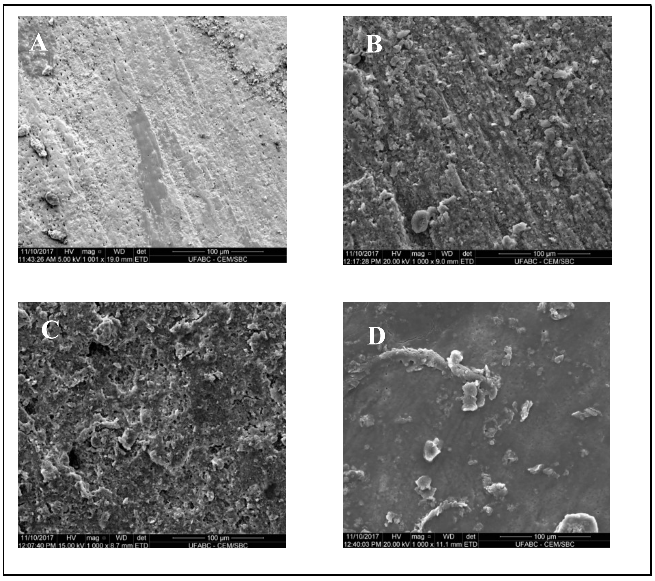

Fig. 7. Eletromicrographs of carious root dentin after submission to a 8-day pH-cycling model. Untreated dentin (A), dentin after treatment with APF-gel (B), dentin treated with Er,Cr:YSGG laser (C) and treated with bioactive glass-ceramic + Er,Cr:YSGG laser (D). Original magnification = 1000 x.

The pH-cycling promoted the opening of dentinal tubules on untreated samples, as well as a suggestive loss of material from the surface (Fig. 7A). The application of APF-gel resulted in a more irregular surface after pH-cycling, in which tubules are partially covered by a material from the pH-cycling solutions. The Er,Cr:YSGG laser resulted in an irregular surface typical from ablation process [15], and this surface appears to have been maintained after pH-cycling (Fig. 7C). This finding suggests that the irradiated surface can more resistant to the pH-cycling model used in this study.

The Er,Cr:YSGG laser irradiation after the bioactive glass-ceramic treatment resulted in a surface smoother than the other experimental groups (Fig. 4D), but with some granules suggestive of biomaterial particles. This aspect indicates that the biomaterial was melted by the thermal action of the laser, and that this was maintained even after the pH cycling.

The values of optical attenuation coefficient evidenced that laser irradiation after the Biosilicato® application significantly decreased these values when compared to all other experimental groups (Fig. 8). Again, this fact suggests that the association of treatments was more efficient in the remineralization of the incipient lesions when compared to the effects of the treatments alone.

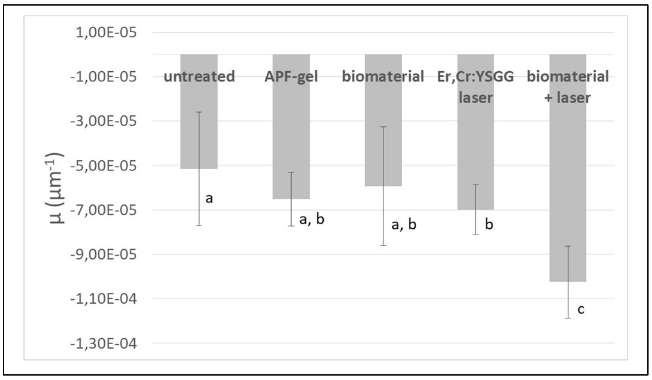

Fig. 8. Means of the optical attenuation coefficients (μ) after 8 days of carious challenge of root dentin untreated, treated with APF-gel, bioactive glass-ceramic, Er,Cr:YSGG laser and glass-ceramic + Er,Cr:YSGG laser. Bars denote standard deviation. Distinct supercript letters indicante statistical differences according to the Tukey´s test ($p < 0.05$).

D. *Q-switched Nd:YAG laser and bioactive glass-ceramic on dentin morphology*

The SEM images observed in Fig. 9 evidences a slight rough surface after irradiation with the *Q-switched* Nd:YAG laser irradiation, with the presence of small microcracks and dentin tubules totally closed (Fig. 9A). These findings agree with previous studies [18] and are suggestive of melting and recrystallization of the surface. Similar characteristics were found when the irradiation was made after the application of the coal paste; however, the irradiated surface was smoother and with less microcracks (Fig. 9B).

The coal paste is widely used in the literature as a photoabsorber [2, 3]; in this way, it increases surface heating and restricts the spread of heat to the deeper tissues. Considering that the Nd:YAG laser photons are not absorbed by the main dentin chromophores [19], the use of the photoabsorber is necessary as a safety measure for the pulp tissue. In this study, the morphological findings agree with this statement and indicate that the use of the coal paste promotes better effects, mainly decreasing the presence of thermal damages such as microcracks.

After applying Biosilicate®, it is noticeable that the dentinal tubules are covered by the biomaterial, which forms a homogeneous layer on the surface of the dentin, although there are some residual particles (Fig. 9C). The irradiation of this biomaterial with the *Q-switched* Nd:YAG laser lead to the formation of an irregular surface with the presence of a few remanescent particles, and an aspect of melting and recrystallization of the material (Fig. 9D).

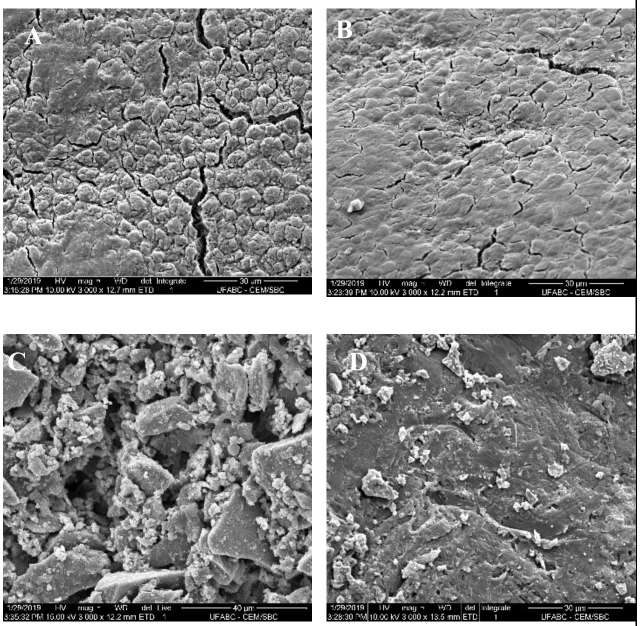

Fig. 9. Eletromicrographs of root dentin treated with Nd:YAG laser without coal paste (A), after treatment with glass-ceramic (B), Nd:YAG laser after coal paste appliaction (C) and glass-ceramic + Nd:YAG laser after coal paste application (D). Original magnification = 3000 x.

This morphological finding is similar to that observed when the biomaterial was irradiated with the Er,Cr:YSGG laser and indicates that the heat generated by the *Q-switched* Nd:YAG laser was also sufficient to melt the material and resolidify it on the surface of the dentin. This is a sign that laser irradiation may increase the retention of the biomaterial against a cariogenic challenge and prolong its effect, in the same way as observed with the Er,Cr:YSGG laser, which will be tested in a future experimental phase.

V. CONCLUSIONS

Infrared lasers are promising for preventing root dental caries and erosion, and the association with fluoride or bioactive glass-ceramic seems to be a better strategy. OCT technique is a powerful method for diagnosing early demineralization and monitoring the effects of treatments on the progress of caries or erosion lesions.

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