Original Research

Laser Irradiation Prevents Root Caries: Microhardness and Scanning Electron Microscopy Analysis

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Abstract

Context: A promising option for the prevention of dental caries is the use of laser irradiation. **Aims:** Evaluate the effects of Er:YAG, Nd:YAG, and CO_2 laser irradiation, associated or not to 2% sodium fluoride (2% NaF), on root caries prevention. **Material and Methods:** One hundred and four human root dentin samples were divided in eight groups (n = 13). A 9-mm^2-area on each dentin sample was delimited and treated as follows: G1: no treatment (control); G2: 2% NaF; G3: Er:YAG; G4: 2% NaF + Er:YAG; G5: Nd:YAG; G6: 2% NaF + Nd:YAG; G7: CO_2; G8: 2% NaF + CO_2. When used, the 2% NaF was applied before irradiation for 4 min. The samples were subjected to a 2-week cariogenic challenge, consisted of daily immersion in de-remineralizing solutions for 6 h and 18 h, respectively. Knoop hardness (KHN) were evaluated (10 g and 20 s) at different depths from the dentin surface. The samples (n = 3) were prepared for scanning electron microscopy (SEM). Microhardness data were analysed by one-way analysis of variance (ANOVA) and Fisher's test ($\alpha = 5\%$). **Results:** The Er:YAG laser group (KHN = 41.30) promoted an increase in acid resistance of the dentin ($P < 0.05$) when compared to all groups. There was no synergism between laser irradiation and 2% NaF application. Morphological changes were observed after irradiation with all lasers; carbonization and cracks were also observed, except when Er:YAG were used. **Conclusions:** Er:YAG laser irradiation can safely increase the acid resistance of the dentin surface of the root, since it promoted a significant increase in surface hardness. The application of 2% NaF did not result in a synergistic effect.

Keywords: *Fluoride, laser, prevention, root caries*

Introduction

The application of preventive dentistry concepts has contributed to the maintenance of an increased number of teeth in the oral cavity of the elderly population.[1] This dental longevity is also a result of increased lifespan and is accompanied by an enhanced frequency of exposed root surfaces due to periodontal diseases, mechanical injury, surgical treatments, or a combination of these factors.[2] As is known, the biofilm accumulation on those exposed root surfaces increases the risk of root caries occurrence.[3]

Concern about this issue becomes greater when we observe that epidemiological studies have shown that the incidence and the prevalence of root caries in elders are high.[4] The knowledge of dental caries, as well as the preventive measures, seems to be the most rational way to control this disease and thus avoid, in the near future, the high prevalence of disease in a population group that becomes increasingly larger.

The use of fluoride has not led to the elimination of dental caries, which affects all age groups and still represents a risk factor for the occurrence of dental fluorosis. Furthermore, the use of fluoride has been widely studied in relation to the enamel surface,[5] but there is a clear lack of studies related to its possible benefits in root dentin. Thus, new techniques and products containing low concentrations of fluoride or not containing fluoride should be studied to prevent the beginning of the progression of root dentin carious lesions.[6]

Studies[7,8] concerning caries prevention using laser irradiation have been made and demonstrated the solubility reduction of dental enamel after irradiation with high-intensity lasers. It is known that a wide variety of laser devices have the property to interact with dental hard tissues because their wavelengths have strong

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interaction with water and hydroxyapatite, two of the main components of the dental hard tissues.[9]

The Er:YAG, Nd:YAG, and CO_2 laser devices have been investigated for preventive purposes and also for cavity preparation due to the mechanism of ablation. It is important that the laser does not ablate the treated surface, or change the tissue morphologically or chemically. Therefore, to achieve the preventive effect, studies have been performed[10] with low energy densities (sub-ablative parameters), and the evaluation with scanning electron microscopy (SEM) images becomes important to verify any morphological alteration.

Given that there is a lack of studies that evaluated the effect of Er:YAG, Nd:YAG and CO_2 lasers on root caries prevention, it is important to conduct studies that evaluate safe and optimal parameters for root dentin irradiation to determine the preventive potential of these devices in that dental hard tissue. The aim of the present study was to analyse the effects of Er:YAG, Nd:YAG and CO_2 laser irradiation, associated or not to 2% sodium fluoride (2% NaF) on root caries prevention and assessed by microhardness test (Knoop hardness (KHN)) and SEM.

Subjects and Methods

After the University of Sao Paulo – Ribeirao Preto School of Dentistry – Ethical Committee authorization (#0009.0.138.000-09), 52 human molars, extracted due to periodontal diseases, were collected and stored in 0.1% thymol solution (pH = 7.0). After cleansing and root planing using a curette, the teeth were stored in distilled water under refrigeration at 4°C. The crowns were separated from the roots at the cemento-enamel junction using a section machine (Minitom, Struers Inc., Westlake-OH, USA) with a diamond disk (Isomet; 10.2 cm × 0.3 mm, arbour size 1/2 in., series 15HC diamond; Buehler Ltd., Lake Bluff, IL, the USA) in low speed. Then, the roots were sectioned and divided in half to obtain 104 fragments of 5 × 5 × 3 mm. The ethics committee of this article was approved in 2009 (University of Sao Paulo – Ribeirao Preto School of Dentistry - Ethical Committee authorization #0009.0.138.000-09).

In addition, a 9.0-mm^2 central area (3.0 mm × 3.0 mm) at the buccal or lingual surface in each one of the dentin samples was delimited. Around this demarcated area, two layers of varnish sealer (Colorama Maybelline Ltda, Sao Paulo, Brazil) were applied.

The samples were randomly divided into eight groups (n = 13) and the delimitated area was treated according to Table 1. Group 1 received no treatment (control). In group 2, a 2% NaF gel was applied to the samples for 4 min and then stored in distilled water at 37°C until the next step of the experiment.

In groups 4, 6, and 8, the 2% NaF was applied before irradiation for 4 min. The samples of the groups 3 and 4; 5 and 6; and 7 and 8 were irradiated with Er:YAG; Nd:YAG and CO_2 laser devices, respectively. To ensure consistent spot size with the hand irradiation, an endodontic file was fixed on the handpiece and kept a determined distance from the surface during the irradiation procedures. The laser parameters used for irradiation are shown in Table 2. The samples were irradiated once in each direction, moving the handpiece slowly horizontally and vertically, to promote homogeneous irradiation and to cover the entire sample area. The irradiation was performed by hand, scanning the dentin surface with a uniform motion for 10 s. The output power was measured with a power meter (TM- 744D, Tenmars Electronics Co. Ltd., Taipei, Taiwan). At the end of these treatments, all samples were kept in distilled water at 37°C until the next step. Afterward, the samples were submitted to a cariogenic challenge.

Table 1: Treatment employed in the different groups

Group	Treatment
G1	Er:YAG laser irradiation
G2	Er:YAG laser irradiation followed by NaF application
G3	NaF application + Er:YAG laser irradiation, simultaneously
G4	Nd:YAG laser irradiation
G5	Nd:YAG laser irradiation followed by NaF application
G6	NaF application + Nd:YAG laser irradiation, simultaneously
G7	NaF application (positive control group)
G8	No treatment (negative control group)

For the cariogenic challenge, samples were submitted to a pH-cycling procedure. The demineralization solution (pH = 4.3) consisted of 2.0 mmol/l of Ca and 2.0 mmol/l of phosphate in buffer solution of acetate 0.075 mol/l, and the remineralization solution (pH = 7.0) consisted of 1.5 mmol/l of Ca, 0.9 mmol/l of phosphate and 150 mmol/l of potassium chloride. First of all, each specimen was immersed in 3.0 ml of demineralising solution for 6 h at 37°C. Then, the specimens were washed with distilled water for 1 min and immersed in the remineralizing solution for 18 h at 37°C. This cycle was carried out for 14 days. At the end of each 5 consecutive days of cycling, the samples were immersed in the remineralising solution for 2 days.

At the end of the pH cycling, 10 samples of each group were sectioned longitudinally through the exposed area. The samples were embedded in epoxy resin, with the cut face exposed. After serially polishing the embedded teeth, each sample was assessed with a microhardness examination of the dentin, starting at 30 μm from the outer surface, with indents at 30 μm, 60 μm, 90 μm and 120 μm. In each area, three measurements of Knoop microhardness were done, and the distance between measurements was 500 μm, to prevent that the marks overlap each other. A static load of 10 g/20 s was applied. The depth of 200 μm was also

Table 2: Lasers parameters of the experimental groups

Parameters	Lasers		
	Er: YAG	Nd: YAG	CO_2
Manufacturer	Kavo Co., Germany	Deka, Italy	Shanghai Jue Hua Laser Tech. Development Co. Ltd., China
Equipment Template	Kavo Key Laser II	Smartfile	PC015-A
Wavelength (nm)	2.940	1.064	10.600
Repetition Rate (Hz)	2	10	20
Beam Diameter (mm)	0.63	0.30	1.00
Irradiation distance (mm)	4	1	8
Output Power	0.6 W	0.5 W	0.2 W
Water Flow	2.0 mL/min	No cooling	No cooling
Irradiation time (s)	10	10	10

examined to certify that the four depth measurements terminate before the end of the lesion.

The preparation of the specimens for the SEM analysis required an initial immersion in 2.5% glutaraldehyde (Merck KGaA, Darmstadt, Germany) with 0.1 M sodium cacodylate buffer solution (pH = 7.4) (Merck KGaA) for 12 h at 4°C. After fixation, the specimens were rinsed with 0.1 M sodium cacodylate buffer solution several times, and then they were sequentially dehydrated in increasing concentrations of ethanol solutions (Labsynth Produtos para Laboratorio Ltda., Diadema, Brazil) as follows: 25% for 20 min, 50% for 20 min, 75% for 20 min, 90% for 30 min and 100% for 60 min, after which they were immersed in a hexamethyldisilane solution (Merck KGaA) for 10 min. Then, they were placed on absorbent paper in glass plates and left to dry under an exhaust hood. The specimens were mounted on metallic stubs with their experimental surfaces face up; they were sputter coated with gold (SDC 050; Bal-Tec AG, Balzers, Liechtenstein), and examined in a scanning electron microscope (Philips XL30 FEG-SEM; Philips Electron Optics, Eindhoven, Holland) operating at 10 kV.

For the microhardness test, first, the assumptions of equality of variances (modified Levene equal-variance test) and the normality of the error distributions (Shapiro–Wilk test) were checked for the response variables tested. Since the assumptions were satisfied, the ANOVA test (α = 5%) was applied using OriginPro 8 SR0 software (Origin Lab Corporation, Northampton, MA). The Fisher LSD multiple comparison test was used at the 5% significance level to evaluate the differences between the means. Qualitative analysis was performed using images obtained with SEM.

Results

Table 3 shows the microhardness mean values (standard deviation) of the experimental groups. Group 3, irradiated with Er:YAG laser, showed an increase in acid resistance and was statistically different from the control group ($P < 0.05$). The other groups showed similar results to the control group. The use of 2% NaF, associated or not with laser irradiation, did not present a tendency to increase the acid resistance of root dentin.

Table 3: KHN mean values (SD) of the experimental groups

Group	Knoop Hardness Number
No treatment (control)	28.65 (3.59)[a]
NaF application	28.50 (4.32)[a]
Er:YAG laser irradiation	41.30 (3.92)[b]
NaF application followed by Er:YAG laser irradiation	29.12 (4.73)[a]
Nd:YAG laser irradiation	25.75 (4.03)[a]
NaF application followed by Nd: YAG laser irradiation	22.65 (3.01)[a]
CO_2 laser irradiation	27.35 (4.12)[a]
NaF application followed by CO_2 laser irradiation	23.90 (2.87)[a]

* Same superscript letters indicate statistical similarity (P>0.05)

The images of SEM are shown in Figure 1a to 1h. The use of Er:YAG laser showed quite satisfactory changes in the irradiated root dentin [Figure 1c], as it presented a more homogeneous dentin, with less irregularities. On the other hand, cracks and carbonization areas were observed in the specimens irradiated with Nd:YAG and CO_2 lasers (Figure 1e and 1g, respectively).

Discussion

The combination of fluoride and laser methods for caries prevention has been extensively studied on the enamel surface; however, there is a lack of studies about their effect on root dentin caries prevention, which was exactly the aim of this study. The results suggested that all the treatments were promising to increase the acid resistance of the root dentin, especially the G3 (Er:YAG), which showed the highest microhardness values after the proposed treatments. The association between laser and fluoride did not produce better results than applying them separately.

Laser irradiation seemed to promote a surface more resistant to caries than the simple application of 2% NaF. It has already been demonstrated that the use of fluoride gel alone is not able to protect the root surface against the attack of acidogenic bacteria.[11] In fact, the results of the present study showed no difference in the comparison between the

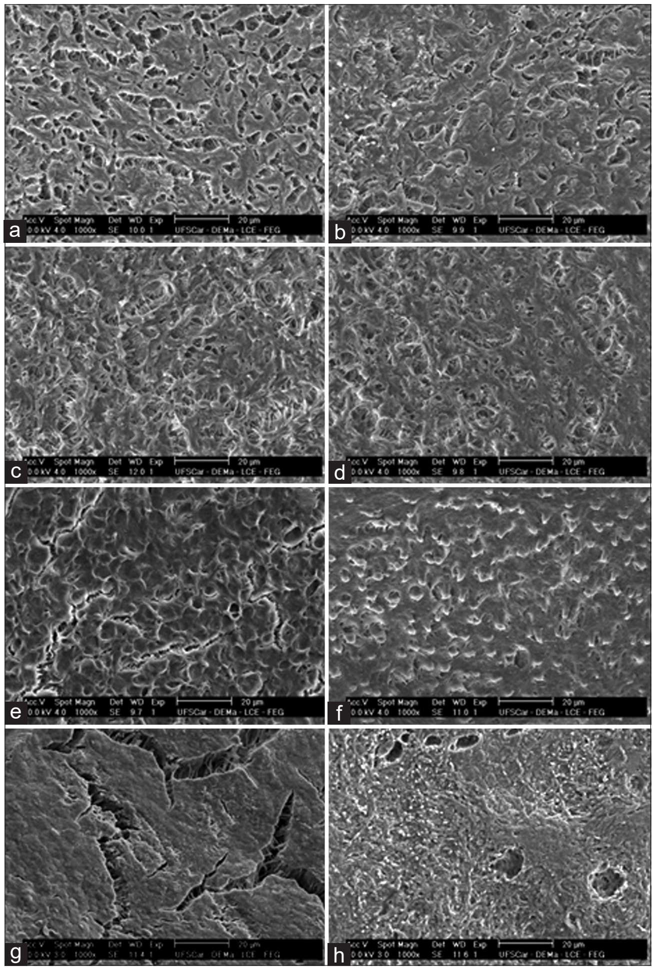

Figure 1: Morphological analysis (×1.000 magnification). (a) G1 - no treatment (control); (b) G2-2% NaF; (c) G3 - Er:YAG; (d) G4 – 2% NaF + Er:YAG; (e) G5 - Nd:YAG; (f) G6-2% NaF + Nd:YAG; (g) G7 - CO_2 and (h) G8 – 2% NaF + CO_2

control and fluoride groups. Moreover, considering that adults and the elderly will constitute the major portion of future societies in many industrialized countries,[12] it makes sense to reflect now on new methods for preventing root caries lesions. In this direction, we studied in the present research the use of three different types of lasers.

The results left us very hopeful about the use of lasers, especially in relation to Er:YAG laser. Similar results were found by Hossain *et al*.,[13] and they emphasized that the Er:YAG lased areas seemed to be thermally degenerated when irradiated without water mist. For this reason, we chose to use in the present study the Er:YAG laser with water cooling. Both studies evidenced that Er:YAG laser irradiation appears to be effective for caries prevention.

In regard to CO_2 laser irradiation, the tubule orifices were obviously occluded but depressed into crates. The association of 2% NaF + CO_2 primarily showed a smooth appearance of surface structure. Our findings are in agreement with a previous study[14] which used a similar methodology in relation to CO_2 laser irradiation.

Al-Omari and Palamara[15] observed that Nd:YAG laser irradiation has resulted in a reduction in dentin microhardness values, even though the specimens were not submitted to the cariogenic challenge after irradiation. Considering that the Nd:YAG laser irradiation, by itself, was unable to maintain hardness values similar to those found in the non-lased dentin, the subsequent cariogenic challenge performed in the present study probably has enhanced the decrease of microhardness and, therefore, this laser has not obtained satisfactory results when compared to Er:YAG laser.

Regarding the association of fluoride, although there were no statistically significant differences in the microhardness among the treated groups, the application of 2% NaF before laser irradiation seemed to promote a more homogeneous and uniform dentin than the laser groups, when just the laser was used.

The mechanism of the synergistic effect between laser irradiation and fluoride is still unknown. During a cariogenic challenge, the loosely bound calcium fluoride (CaF_2) may liberate the fluoride ions to inhibit demineralization and enhance remineralization.[16] The firmly bound fluoride integrated into the crystalline structure may increase crystal stability and acid resistance. Probably this also occurs in dentin. In addition, the firmly bound fluoride may serve as a fluoride reservoir, with a greater substantivity than that of the loosely bound fluoride.[16] It is important to note that, in the present study, 2% NaF was applied before irradiation. Thus, further studies are needed to determine whether the same effects would occur if the fluoride were applied after the use of a laser.

Another hypothesis to explain why lasers can increase the acid resistance of the dental enamel was proposed by Hsu *et al*.,[17] who suggested the "organic blocking" theory, when the partial denaturation of organic matrix caused by laser irradiation may block the diffusion pathway in enamel, resulting in a retardation of enamel demineralization. Blocking the diffusion pathway may affect the enamel's porosity and micro surface area. The organic matter, causing a statistically significant decrease in the pore volume and surface area in enamel after laser irradiation, may be one of the key players in the laser-induced blocking of the diffusion pathway and subsequent prevention of enamel demineralization.[18] If this organic blocking theory could be extrapolated to the dentin surface, these studies would support the results obtained in the present study.

When water cooling is not used, the dentin undergoes a melting process that involves, among other things, the obliteration of the dentinal tubules.[19] This hinders the penetration of acids into the dentin, making this tissue more resistant to demineralization. However, in the present study, cracks and small spots of carbonization on the root dentin were observed when the specimens were irradiated with Nd:YAG and CO_2, probably because these lasers were used without water cooling.

These considerations about water cooling are of extreme importance because it indicates that higher fluencies than that used here would cause significant thermal damage in irradiated dentin. Furthermore, higher fluencies could lead to dentin ablation and also induce a greater mineral loss during an acid challenge.[20] This mineral loss probably occurred in the present study, once we observed lower values of microhardness in the groups irradiated with Nd:YAG and CO_2 than Er:YAG irradiated dentin.

Therefore, analysing the results obtained in the present study, it was possible to confirm, after the microhardness test and SEM evaluation, that the Er:YAG laser promoted an increase in acid resistance of the human root dentin, without causing carbonization or cracks. Furthermore, the application of 2% NaF did not result in a synergistic effect when combined with laser irradiation, in relation to increasing the acid resistance. At this point, new studies should be developed to understand how the irradiated tissue becomes more resistant to acids and to verify possible morphological and structural changes caused in the root dentin after laser irradiation.

Conclusion

The results suggest that Er:YAG laser irradiation can safely increase the acid resistance of the human root dentin surface since it promoted a significant increase in surface hardness without causing carbonization or cracks in the tooth structure. Furthermore, the data showed that the application of 2% NaF did not result in a synergistic effect when combined with laser irradiation with regard to increasing acid resistance.

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Conflicts of interest

There are no conflicts of interest.

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