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ORIGINAL ARTICLE

Progression of erosive lesions after Nd:YAG laser and fluoride using optical coherence tomography

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Abstract This study aimed to use optical coherence tomography (OCT) to assess the progression of erosive lesions after irradiation with Nd:YAG laser and application of topical fluoride. One-hundred and twenty dentin samples (4 × 4 × 2 mm) obtained from bovine incisors were used. Samples were protected with acid-resistant nail varnish, with exception of a central circular area 2 mm in diameter. All samples were submitted to erosive cycles with citric acid solution 0.05 M (citric acid monohydrate—C6H8O7·H2O); M = 210.14 g/mol) pH 2.3, at room temperature, for 20 min, 2×/day, throughout 20 days. After 10 days of acid challenges, lesions became visible, and each group received a different treatment (n = 15): control (without treatment), topical application of sodium fluoride 2 % for 4 min; Nd:YAG laser with different irradiation parameters (1, 0.7, and 0.5 W); and the association of fluoride with the laser parameters. OCT readouts were performed on day 01 (before the first acid challenge—OCT1), on day 05 (OCT2), day 10 (OCT3—after treatment), day 15 (OCT4), day 17 (OCT5), and day 20 (OCT6). The OCT images generated made it possible to measure the amount of tooth tissue loss over the 20 days of erosive cycle, before and after treatments, and to monitor early dentin demineralization progression. After statistical analysis, the fluoride group was observed to be the one that showed smaller loss of tissue over time. The OCT technique is promising for diagnosing and monitoring erosive lesion damage; however, further in vitro and in vivo research is needed to improve its use.

Keywords Dentin · Erosion · Nd:YAG laser · Fluoride · Optical coherence tomography

Introduction

In recent decades, a substantial and global decline in the prevalence of dental caries has been observed. Early and constant accomplishments of dentists, especially with regard to educating patients, alerted the population about the importance of correct tooth brushing and appropriate habits for maintaining oral health. Moreover, over the years, topical fluoride application for preventing dental caries has been primarily responsible for reducing the incidence of caries in the population, together with the control of plaque and periodontal therapies [1–3].

Also, life expectancy of the population has increased [4, 5]. These facts resulted in the appearance of other conditions such as the non-carious cervical lesions (NCCL). There is a consensus that NCCL result from the interaction of several factors simultaneously.

The diagnosis and the choice of treatment depend on correctly identifying these factors and the associated presence or absence of painful symptoms [4–6].

Among the NCCL, erosion is characterized by loss of tooth structure due to a chemical process, without bacterial

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involvement, leading to an irreversible loss of dental hard tissue and progressive softening of the surface, making the areas affected most susceptible to the action of abrasive processes. There is high prevalence and incidence of these large, flat, saucer-shaped lesions, without formation of sharp angles. Intrinsic (gastro-esophageal reflux, bulimia, duodenal ulcers, recurrent vomiting by alcoholism) and extrinsic acids (diet, medications, environment) are one of the many causes of these lesions [7–12].

Many studies in the literature have been focused on the prevention of erosive lesions in enamel with laser [13–17]. However, clinically, most patients already have stablished lesions when they arrived at dental offices, requiring guidance and treatment to control or avoid the progression of these lesions. This requires a multidisciplinary approach that may include nutritional education, psychological care, application of fluoride, and laser therapy.

Laser irradiation is able to increase acid resistance by structural modification achieved by the thermal effect produced on both enamel and dentin, with loss of water and carbonate, and incorporation of fluoride into the tissue structure. This results in fluorapatite that is less soluble and formed by larger and more stable crystals than hydroxyapatite [13, 14]. Nd:YAG laser is considered as a promising laser equipment for increasing the acid resistance of dentin, but still lacks safe protocols for prevention or interruption of erosive lesion progression [13–17].

As erosive lesions have a multifactorial etiology, their diagnosis is made through meticulous oral clinical examination and detailed study of the patient's medical history, with special attention to nutritional assessment, occupational health, and eating habits [7, 8, 18]. In vivo, there are no devices for early diagnosis and quantification of tissue loss in erosive lesions. Usually, study models, silicon indexes, and photographs are used at regular intervals to clinically monitor the progression of lesions [18].

One useful tool to analyze erosive lesion progression would be the optical coherence tomography (OCT). It is a non-contact, non-invasive, and non-destructive technique, with non-ionizing radiation, that could detect a signal of backscattered light from different depths of a tissue sample allowing its use in vivo. OCT produces real-time, high spatial resolution, transverse section images.

Since there is not a consensus about the correct laser parameter for the prevention of erosive lesion progression and a method for diagnose and monitor these lesions, the aim of this in vitro study was to evaluate different parameters of the Nd:YAG laser, topical fluoride application, and their association in the progression of erosive lesions, by using OCT as a possible technique for diagnosing and monitoring lesion progression in dentin.

Materials and methods

Preparation of samples

For the development of this in vitro study, 120 single-rooted bovine teeth were used. Dentin squares measuring 4 × 4 × 2 mm were obtained from the cervical region, resulting in 120 samples that were stored in distilled water. Samples were embedded in acrylic resin and polished with abrasive papers to standardize the surface.

At the center of each sample, an adhesive disk 2 mm in diameter was inserted, corresponding to the area of the erosive lesion to be formed. A dental drill was used to make a groove in the opposite edges of the samples, indicating the direction of the OCT readout (OCT1), making it possible to repeat all the readouts in the same region.

All surfaces were covered with an acid-resistant nail varnish. After drying, the adhesive disks were removed, surfaces were cleaned with deionized water, and acid challenge was carried out. Samples were labeled individually and divided into 8 groups (1. control; 2. laser 1/L1; 3. laser 2/L2; 4. laser 3/L3; 5. fluoride/F; 6. fluoride + laser 1/F+L1; 7. fluoride + laser 2/F+L2; 8. fluoride + laser 3/F+L3), stored and maintained in deionized water.

Erosive challenge

To induce dentin erosion in vitro, samples were inserted in a solution of 80 ml of 0.05 M citric acid (citric acid monohydrate—$C_6H_8O_7 \cdot H2O$, M = 210.14 g/mol) with pH 2.3; at room temperature for 20 min; washed in water for 15 s, with a 12-h interval for 5 days. Samples were then analyzed by OCT (OCT2), and no erosive lesions were observed. For this reason, they were subjected to an additional 5-day period of acid challenge, and a new OCT readout (OCT3) was performed. Erosion lesions were visible, and samples were ready to receive the treatments.

Laser irradiation

An Nd:YAG laser with wavelengh of 1064 nm (Power Laser™ ST6, Chico, CA, USA) was used with the following parameters: power of 0.50 W (L1), 0.70 W (L2), and 1 W (L3), adjusted with a power meter (Coherent, Newport, USA); repetition rate of 10 Hz, 120 ms of pulse width, fiber optic of 400 μm. Irradiations were performed in contact mode, perpendicular to the surface, with scanning movements. Four irradiations of 10 s were performed, totaling 40 s of irradiation, covering the entire lesion surface, with an interval of 10 s between irradiations, for thermal relaxation. Table 1 describes the laser protocols used.

Table 1 Description of laser irradiation protocols

GROUP	Power (W)	Repetition rate (Hz)	Time (s)	Energy (mJ)	Energy density (J/cm^2)
L1	1.0	10	10	100	79.57
L2	0.70	10	10	70	55.70
L3	0.5	10	10	50	39.78

Topical application of fluoride

Groups F, F + L1, F + L2, and F + L3 received application of sodium fluoride 2 % (GEL Flutop Neutral-SS White, Rio de Janeiro, RJ, Brazil) for 4 min. After this, samples were rinsed with distilled water for 10 s and dried with paper towels. Then, laser irradiation was perfomed, in accordance with Table 1.

Evaluation of erosion progression with OCT

The OCT system used in this study was a OCP930SR Model (Thorlabs Inc., Newton, USA), with a light source consisting of a super-luminescent light-emitting diode (SLED), lateral and depth resolution of 6.0 microns in air, and 3 fps (2000 × 512 pixels).

After treatments on day 10, samples were subjected to a new acid challenge, in the same way as described before, for 10 days. New OCT analyses were performed after 05 days (OCT 4), 07 days (OCT5), and 10 days (OCT6) in order to assess tissue loss.

The OCT sweep was performed tangential to the unprotected area. At the geometric center of the region of analysis, a cross-sectional grayscale image was generated. The cross-sectional images were used to calculate the loss of dentin and to assess changes in the signal received due to demineralization.

One hundred and twenty images were generated by OCT analysis per day (total of 720 images of OCT1 to OCT6) and evaluated according to their reference surfaces, before acid challenge and different treatment methods. Thus, each sample had its own control area.

A central area was selected to standardize the site where the dentin loss was calculated: the lateral limits for measuring the lesions was the lateral sides of this selected area and the lower limit was the exposed surface contained within the demarcated area. Thus, a standard area was obtained in all images allowing comparison between all the groups.

Measurement was performed using ImageJ software, manually outlining the limits of the selected area on a touchscreen notebook, with the use of an appropriate pen. Fifty measurements of an image with many irregularities were chosen to determine the reliability of manual selection. The variation between measurements was approximately 5 % (operational error).

ImageJ software automatically generates tables in Microsoft® Office Excel® 2007 software from the data obtained, and from these, graphs were created for better visualization of variations. It was possible to observe dentin loss graphs for each sample after the 20 days: graphs of the variation in the arithmetic mean of all the 15 samples in each group according to time and graphs of the area of lesion progression normalized by the average.

OCT images allowed assessment of the tissue loss and the increase in the white band (backscatter radiation) related to the demineralization that occured. The increasing depth of penetration of the OCT signal into the sample indicated the demineralization progression over time.

Measurements were made of the white band to demonstrate the possibility of longitudinal follow-up over time (qualitative analysis) for illustrative purposes, and not with the intention of obtaining actual measurement values (quantitative analysis).

Results

Two-way analysis of variance (ANOVA) (two factors—treatment and time of exposure to acid) was used to assess the progression of erosive lesions. The normal distribution of samples was determined from the results obtained after performing the Kolmogorov-Smirnov test.

The eight groups were evaluated to assess the area affected (control, fluoride, fluoride + laser1, fluoride + lasers2, fluoride + laser3, laser1, laser2, and laser3). Measurements were obtained in the following time intervals: 0, 5, 10, 15, 17, and 20 days. Table 2 shows the data of area (pixels) according to time and the treatment.

The ANOVA model included the effect of two factors studied (treatment and time) and their interaction (treatment * time). The result of the interaction indicated that the change in the affected area over time depended on the treatment groups, and similarly, the change in area between treatments depended on time.

For data analysis, Minitab statistical software (version 16.0) was used. The level of significance was set at $p < 0.05$. Results showed an effect of interaction between the factors "time" and "treatment," statistically significant ($F = 4.20$, $p < 0.001$). This interaction indicated that the increase in the area of lesion measured over time was not the same for all treatment groups. Furthermore, the difference between the measured area of the treatment groups could vary over time. Since there was the effect of interaction, the areas between treatment groups were compared separately for each time

Table 2 Summary measures of the area (pixels) according to time and the treatment

Time	Treatment	*n*	Mean	SD
0	Control	15	501	1.6
0	Fluoride (F)	15	552	169.9
0	F + laser 1	15	569	260.2
0	F + laser 2	15	502	1.9
0	F + laser 3	15	501	1.8
0	Laser 1	15	498	1.7
0	Laser 2	15	562	172.0
0	Laser 3	15	497	1.2
5	Control	15	5417	2629
5	Fluoride (F)	15	5125	1959
5	F + laser 1	15	5536	1979
5	F + laser 2	15	4201	1663
5	F + laser 3	15	5090	2055
5	Laser 1	15	4359	1389
5	Laser 2	15	4796	2099
5	Laser 3	15	6726	3158
10	Control	15	10,338	4469
10	Fluoride (F)	15	9818	4996
10	F + laser 1	15	9486	3830
10	F + laser 2	15	7156	2233
10	F + laser 3	15	8843	2934
10	Laser 1	15	8626	2277
10	Laser 2	15	7902	2539
10	Laser 3	15	11,139	4928
15	Control	15	15,090	8476
15	Fluoride (F)	15	12,674	7950
15	F + laser 1	15	26,093	5569
15	F + laser 2	15	20,681	7367
15	F + laser 3	15	19,115	10,256
15	Laser 1	15	27,366	8157
15	Laser 2	15	24,800	7751
15	Laser 3	15	23,771	7390
17	Control	15	16,198	9338
17	Fluoride (F)	15	12,336	8097
17	F + laser 1	15	26,066	5541
17	F + laser 2	15	20,634	6483
17	F + laser 3	15	19,616	9502
17	Laser 1	15	28,161	9197
17	Laser 2	15	23,894	6383
17	Laser 3	15	24,481	7634
20	Control	15	18,907	9478
20	Fluoride (F)	15	14,137	8095
20	F + laser 1	15	29,110	5070
20	F + laser 2	15	22,209	5992
20	F + laser 3	15	19,201	7720
20	Laser 1	15	30,832	8942
20	Laser 2	15	22,554	7078
20	Laser 3	15	26,141	8652

interval. The Tukey multiple comparison test was used to verify, at each time interval, which group showed significant differences.

In general, it was noted that the area increased progressively until day 15 and remained constant until day 20. This finding was also proved by the method of Tukey multiple comparisons.

Different effects were observed for treatments performed in vitro to initially prevent erosion lesion progression in dentin samples. The control group showed an increase in tissue loss over time, as expected, due to the sequence of chemical cycling, without any type of treatment performed. When fluoride was associated with laser, the results were superior to those obtained with laser used alone, with the exception of group F + L1. The fluoride group was the one that demonstrated the greatest ability to prevent the progression of erosion lesions ($p < 0.05$) in days 15 and 17.

There was no statistical difference between any of the groups until treatments were performed ($p > 0.05$), showing standardization of the erosive cycle for all samples. The Tukey test identified significant differences between groups after treatments.

With regard to the laser groups, only the L2 (0.7 W) and F + L3 (0.5 W) protocols were observed to show a decrease in erosion lesion progression, however, not statistically significant. The Tukey test showed that on day 15, there was no statistically significant difference between the laser groups and the laser associated with fluoride groups in the three protocols used. However, on days 15, 17, and 20, the fluoride + L3 group (protocol 0.5 W) presented the lowest loss of dentin, when compared with the other protocols (0.7 and 1 W).

Considering the increase in depth of areas on day 15, after the treatments, the increase in the areas was observed to be more balanced across all groups, compared with the increase that occurred between day 10 and day 15, when irradiated groups (F + L1, F + L2, F + L3, L1 L2, and L3) showed a greater increase when compared with the control groups and fluoride group.

It was possible to estimate a percentage increase on days 15, 17, and 20 and verify the better performance of groups L2 and L3 + F, with results matching those obtained by the Tukey test.

Figure 1 shows the lesion progression of sample 12, treated with fluoride and laser2. Graph 1 illustrates the behavior of lesion progression for all groups.

Discussion

Among the available high-power lasers, the Nd:YAG laser is considered as the "gold standard" laser for the treatment of dentin hypersensitivity, and its clinical effectiveness was already demonstrated in several investigations. Since dental

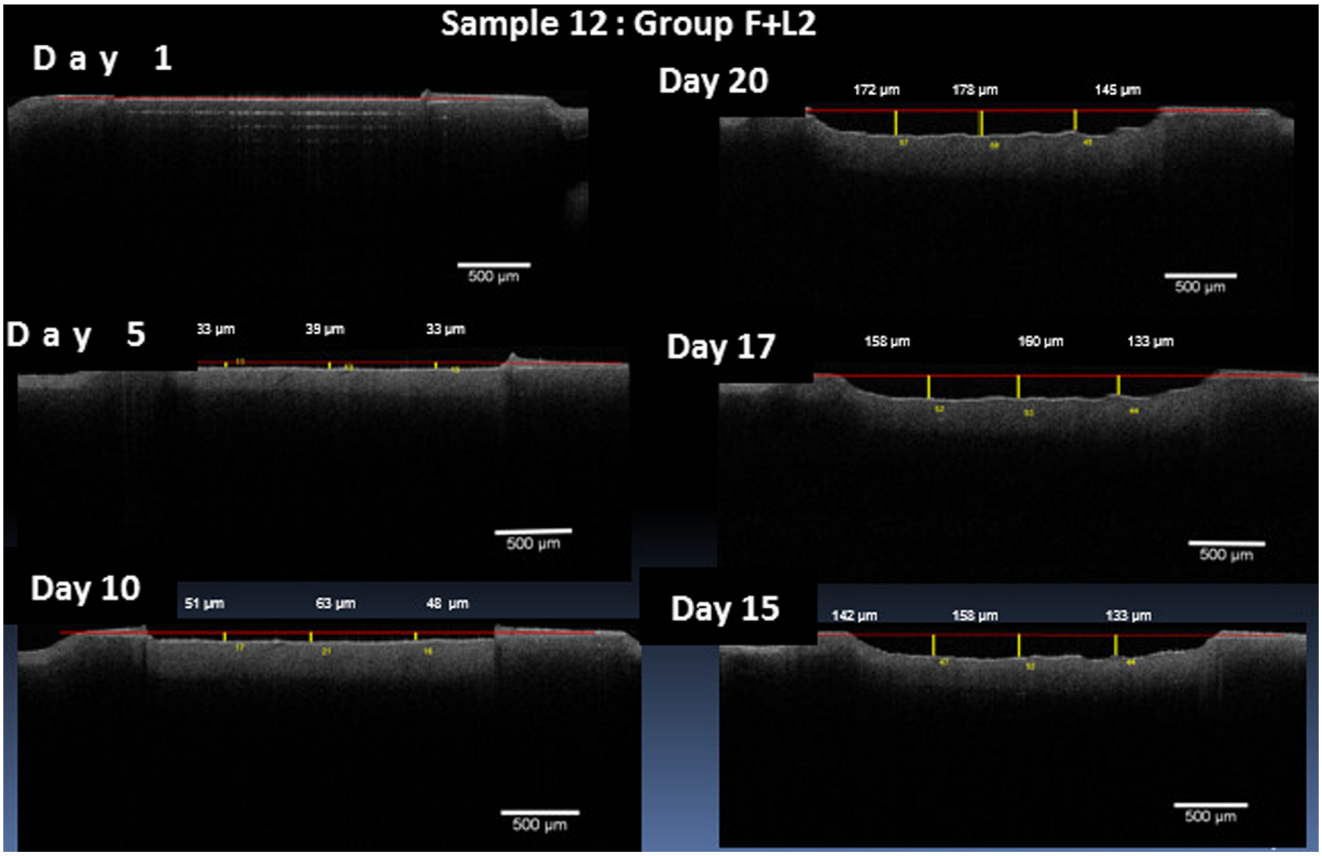


Fig. 1 The lesion progression of sample 12

erosion and dentin hypersensitivity are often related, it would be interesting if the Nd:YAG laser could not only treat dentin hypersensitivity by sealing the dentinal tubules, but also control the progression of dentin erosion, by increasing the acid resistance and modification of the dentin structure.

So, the aim of this study was to evaluate different parameters of Nd:YAG laser, fluoride, and their association in the progression of erosive lesions using OCT as a possible technique for diagnosing and monitoring lesion progression in dentin.

Dental tissue demineralization by acids only starts after intimate contact with the surface. In dentin, the organic layer hinders diffusion of the acid into the deepest regions and has a protective buffer capacity against the acid [10]. However, the literature shows that the action of acids, whether intrinsic or extrinsic, may have greater influence on dentin than on enamel due to its lower inorganic and more organic composition, with a higher percentage of carbonates [19]. Hara [20] and Lussi [21] showed that progression of erosive lesions can be significantly influenced by the following: the type of substrate, chemical and mechanical challenges, fluoride exposure, and contact with the soft tissues of the oral cavity and tongue. In this context, this in vitro study did not simulate the oral condition itself, and for a better analysis of lesion progression, some factors were removed to standardize the condition and acid challenge. In addition, samples were stored in distilled water and not in artificial saliva, to avoid the possibility of a remineralization process that could interfere with the final results.

Samples were standardized and not subjected to ultrasonic cleaning to remove the smear layer formed, which may explain the initial difficulty in detecting significant erosive lesions in the beginning of the erosive cycle. The smear layer presented after root scaling exposes dentin to the oral environment and acts as a barrier to the demineralizing action of acid substances. In the present study, initially, an acid challenge for 5 days with citric acid was proposed. However, after the first 5 days, it was noted that little visible change had occurred. Thus, another 5 days of challenge (10 days total) was performed. After 10 days of the chemical erosive cycle, erosive lesions with typical characteristics were observed by OCT.

According to data obtained, it was possible to verify that none of treatments performed was able to prevent the progression of erosive lesions against acid challenge. It is hypothesized that at pH below 4.5 (critical for erosion), dissolution of carbonated apatite, fluoridated hydroxyapatite, and fluorapatite occurs.

On the other hand, other studies have found evidence of increased acid resistance of dentin with Nd:YAG laser irradiation alone or associated with fluoride. This increase in acid

resistance promoted by Nd:YAG laser has positive clinical effects, already proven in the literature in regard to dental caries prevention. Recently, the effect of laser irradiation with this wavelength was studied for prevention and/or treatment of erosive lesions [15–17].

In the present study, it was possible to observe the lowest increase in the area measured in the fluoride group at the end of the experiment, indicating that this was the group with the lowest dentin loss. The protective effect of fluoride has been described in several studies for promoting an increase in the acid resistance of the treated tissues [4], but most authors seemed to agree with the fact that the CaF_2 layer formed can easily be removed by acidic drinks, and the benefits of fluoride application alone would be quickly neutralized [7]. To be considered effective, frequent applications would be required [22]. Lussi et al. [21] alerted to the fact that although the majority of toothpastes currently being used worldwide are fluoridated, the incidence of erosive lesions continues to increase over time, meaning that even with the wide range of fluoride products available to populations, and the importance of lower caries prevalence, there is an increasing trend toward the formation of erosive lesions over time [23].

With respect to the Nd:YAG laser protocols in all the irradiated groups, there was an increase in lesion area immediately after irradiation, especially in groups L1 and F + L1, in which the higher power setting (1 W) was used. After Nd:YAG laser irradiation with 1 W power in the contact mode, surfaces became irregular with destruction of edges of the acid-resistant varnish layer. So, it can be concluded that the use of Nd:YAG laser in contact mode can cause the formation of craters [15–17, 24].

When used in non-contact mode, the distance from the fiber to the sample surface can cause a decrease in energy density (ED = Epulse/A; determines the effects of radiation on tissue) and power density (PD = P/A; determines the possibility of thermal damage in the tissue) delivered to tissue, because both magnitudes are inversely dependent on irradiated area. When laser is distanced from the target tissue, the irradiated area increases. Therefore, studies using the same equipment and similar protocols, but in non-contact mode, should be performed. Clinically, erosive lesions in dentin are more hardened than those in experimental samples that have a more resilient and soft surface [21]. These craters may explain why the groups in which laser was used had a final area larger than all the groups, including the control group in which no treatment was performed. However, by assessment of lesions from day 15, the increase in area in the irradiated groups was observed to be more gradual, consistent with the increase in the control and fluoride groups.

When the increase in percentage of area was calculated on the following days after treatment, it was observed that control group presented larger areas, followed by L1 group. In groups F and F + L2, the percentage increase of lesion areas was smaller than in other groups. In groups F + L3 and L2, the areas decreased over time, probably due to a small difference in measurement site on different days. For future studies that will use OCT, readout of three different locations on samples is suggested.

Magalhães et al. compared the efficacy of application to dentin of the following: APF gel; fluoride varnish; Nd:YAG contact laser irradiation with parameters of 0.5, 0.75, and 1.0 W; Nd:YAG laser at 0.75 W associated with FFA; and Nd:YAG laser associated with fluoride varnish [15]. Superior results were obtained with the group treated with FFA, corroborating the results of the present study. The laser was not effective within the parameters used, and the authors suggested that the occurrence of craters by use in contact mode made the surface more susceptible to the action of acids, as occurred in the present study [15].

In this present study, the progression of tissue loss in all groups in the latter half of the experiment was smaller than on the first days of acid challenge, showing that the protocols used should be further investigated, with different settings, mode of operation, and irradiation. Moreover, a preliminary lesion would be recommended to simulate the real condition of a patient with an existent erosive lesion.

With regard to OCT, in this study, erosive lesions were assessed by the equipment and technique, without the need of sample destruction, allowing them to be monitored throughout the 20 days of the experiment. The acquisition speed of the equipment used in the experiment—3 fps—enabled the capture of in vivo images, and up to 15 fps can be used. Even if a patient does not remain completely immobile at the time of image acquisition, image capture will not be impaired. OCT has many advantages over other existing techniques to assess changes affecting dental tissues, such as high-resolution image capture, real-time imaging and non-contact, non-destructive mode, use of non-ionizing radiation, and allowing the diagnosis of early lesions before the appearance of clinical signs [25, 26]. In this study, the increase in the OCT signal could be visualized as early as the second readout (OCT2) before clinical perception of erosive lesions (from OCT3). Mineral loss alters the optical properties of dental tissues, such as scattering, reflection, absorption, and fluorescence spectroscopy, by the emergence of pores and greater incorporation of liquid [27–32].

The use of OCT also showed promise for monitoring erosion lesions in dentin. Manesh et al., in 2008, used a PS-OCT device for evaluating the progression of demineralization caused by caries lesions in dentin after fluoride and Er:YAG lasers [33]. The laser was not effective for increasing or decreasing the rate of dentin demineralization, but the PS-OCT succeeded in measuring

demineralization in the surfaces of samples and verifying inhibition of demineralization by fluoride, in addition to measuring the reflectivity of the area irradiated with lasers. The results obtained by PS-OCT were favorably compared with the results of analysis by PLM and TMR. In a subsequent study, the authors used the OS-OCT to test the effectiveness of anti-caries agents, including fluoride and irradiation with lasers (Er:YAG, Nd:YAG, and CO_2 TEA) in dentin [33]. The technique has been successfully recognized for measuring the inhibition of demineralization.

As previously described, in the early days of acid challenge, erosive lesions were not visible to the naked eye, and even during the analysis with OCT, so that it was necessary to increase the acid challenge time from 5 to 10 days. However, when comparing the images of each sample sequentially, and analyzing the extent of the demineralized range, the increase was easily perceived, which reinforces the advantage of OCT in detecting early changes in the optically derived images of dental tissue demineralization.

The OCT equipment used showed to be extremely important and useful; however, it is necessary to standardize the site of tooth structure loss where measurement must be performed.

By means of OCT, the F + L3 group was observed to present the lowest increase in demineralization progression, consistent with results found in the analysis of lesion progression. These results point to a better performance when F is associated with lower laser energy densities, as has been found in the results of other studies [15, 17].

The L1 group, in which there was a greater loss of tissue and larger demineralized range at the end of the experiment, showed a lower progression of demineralization over time. Despite the formation of craters, the melted surface seems to act as a barrier to acid diffusion. It is possible that cracks formed by laser irradiation with higher power settings allowed the diffusion of acids, despite the melted surface.

The F group showed an increase in the rate of demineralization progression between 17th and 20th days, probably due to poor stability of CaF2 crystal deposits formed that were removed in the presence of acid [12, 34].

Other protocols that analyze the prevention of erosive lesions should be studied, with differences in the mode of Nd:YAG laser irradiation and associations with other products and technologies. It is also important to emphasize that prevention of any disease presents a better cost benefit than treating a disease already existent. Early identification of risk factors (chemical, behavioral, and biological) and appropriate intervention, including behavioral changes of the patients, are essential in preventing damage from erosion, so that therapeutic and preventive measures will be successful over time.

Conclusions

Within the limitation of an in vitro study, it could be concluded that:

- None of the proposed treatments was able to prevent the development of erosive lesions;
- The group treated with fluoride had the lowest dentin loss at the end of experiment. Laser groups with lower power settings showed more satisfactory results;

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest. Authors disclose all relationships or interests that could have direct or potential influence or impart bias on the work.

Ethical statement The manuscript has not been submitted to more than LIMS for simultaneous consideration. The material has not been published previously (partly or in full). This study is not split up into several parts to increase the quantity of submissions and submitted to various journals or to one journal over time. No data have been fabricated or manipulated (including images) to support our conclusions.All authors agree to submit, and they have explicitly signed an agreement document. Authors whose names appear on the submission have contributed sufficiently to the scientific work and therefore share collective responsibility and accountability for the results.

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