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EFFECT OF DIFFERENT FLUORIDES COMBINED WITH ER: YAG LASER TO CONTROL THE PROGRESSION OF DENTIN EROSION

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ABSTRACT

This in vitro study aimed to evaluate the effect of the association of different types of fluorides with Er:YAG laser irradiation on demineralization of the eroded dentin. Slabs of bovine dentin (3x3x2mm) were submitted to 4 erosive challenges in orange juice (pH=3.84) for 5 minutes, 2x/day, during 2 days. After cycles, slabs were assigned into four groups, according to surface treatment: I. placebo gel-control + Er:YAG laser; II. titanium tetrafluoride gel + Er:YAG laser and III. sodium fluoride gel + Er:YAG laser. After surface treatment, new erosive challenges were performed following the same protocol. The microhardness analysis was performed after erosion-like lesion formation, after treatment and after subsequent erosive challenges. Data were submitted to analysis of variance (ANOVA) followed by DUNCAN test, at significance level of 5%. After subsequent erosive challenge, TiF_4 + Er:YAG laser (121.1 ± 3.7) and NaF + Er:YAG (110.8 ± 3.4) laser showed significantly higher microhardness values, followed by the group Placebo + Er:YAG laser (106.2 ± 3.9). The use of titanium tetrafluoride gel and sodium fluoride gel, associated with Er:YAG laser can be an alternative to control demineralization of the eroded dentin.

KEYWORDS: Erosion, titanium tetrafluoride, sodium fluoride, demineralization, laser & microhardness.

INTRODUCTION

Dental erosion is defined as acid-related loss of tooth structure, without the involvement of microorganisms.[1] Due to the high incidence of erosive lesions,[2] much effort has been made to establish a treatment that prevents the dissolution of the dental mineral substrate, increasing the tooth´s resistance to acidic substances.[1]

Therapies based on the application of fluoride compounds have been carried out.[3-9] Sodium fluoride (NaF) can act as a physical barrier through the formation of a calcium fluoride layer. This layer hinders the contact of acid with the tooth structure, or act as a mineral reservoir.[10] Recent studies have focused on other fluorides, such as titanium tetrafluoride (TiF_4),[3-6, 11] that contain polyvalent metals which may have a higher efficacy than NaF due its ability of precipitation or incorporation of ions into eroded dentin.[7, 8]

The mechanism of action of TiF_4 has been attributed to an interaction of titanium with the tooth surface proteins.[12] Pretreatment with TiF_4 produces a titanium coating on the surface[13] which can alter the micromorphology of dentin, producing a precipitate surface layer on intertubular and intratubular dentin, becoming this substrate more resistant to erosive challenges.[14] Due to its ability of sealing dentinal tubules,[13] it has been reported that TiF_4 can reduce dentin hydraulic conductance,[14] as well as, the hypersensitivity.[15]

It has been suggested that TiF_4 could be used before bonding procedures to promote a more stable hybrid layer,[3, 11, 16] however, its effects have not been totally clarified. Tranquilin et al showed that TiF_4 promoted higher immediate bond strength to dentin, regardless which conventional adhesive system was used.[11] On the other hand, according to another study, the bond strength of the composite was not affected by this system,[16] as well as, its use was not effective in inhibiting demineralization around the restoration interfaces.[3]

Fluoride application is the most used treatment to prevent dental erosion,[3-9, 17, 18] however, its effectiveness in reducing erosion has been questioned when it is used alone.[19- 21] The deposited calcium fluoride-like material (CaF_2) from topical fluoride application is supposed to dissolve readily in most acidic drinks, [20, 21] being not able to protect against initial erosive challenges.[19]

Given that the effectiveness of fluoride employed alone as a symptomatic therapy for erosion is still under debate, new preventive therapies have been assessed.[22] Er:YAG laser irradiation promotes structural modifications achieved by a thermal effect produced in enamel and dentin, with water and carbonate loss.[23] Its irradiation may increase the acid resistance of the enamel[24, 25] and dentin.[26] Morphological analysis of eroded dentin showed that Er:YAG laser promoted a regular flat surface with obliterated tubules and collapse aspect of the collagen fibers. However, Er:YAG laser with low energy densities appears to be an alternative for tooth pretreatment, without compromising the adhesion of restorative materials.[27]

When associated with fluoride compounds, such as silver diamine fluoride, Er:YAG laser was able to increase its uptake at depths of up to 20 µm[28] even when it was compared with other lasers at sub-ablative levels.[29] On eroded dentin, the treatment with fluoride varnish combined with Er:YAG laser promoted an irregular appearance and the tubules partially or completely obliterated.[30]

Considering that literature is lacking information about the used Er:YAG laser combined with fluoride compounds in the progression of demineralization of the eroded dentin, this study aimed to evaluate in vitro the influence of the association of different types of fluorides (NaF and TiF_4) with Er:YAG laser irradiation on demineralization of the eroded dentin. The null hypothesis is that the treatment with NaF or TiF_4 combined with Er: YAG laser has no influence on microhardness of the eroded dentin

MATERIALS AND METHODS

Experimental design

The factor under study was the *surface treatment* at 3 levels: placebo gel-control + Er:YAG laser (Placebo + Er:YAG laser), titanium tetrafluoride gel + Er:YAG laser (TiF_4 + Er:YAG laser) and sodium fluoride gel + Er:YAG laser (NaF + Er:YAG laser). The experimental units were composed of 30 bovine dentin slabs (n=10), randomly assigned into 3 groups. The study was a randomized complete block design and the response variable was Knoop microhardness (KHN). The flow chart of the study is presented in Figure 1.

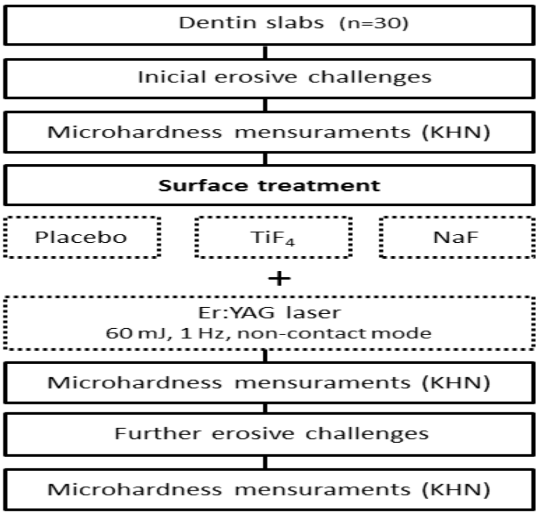

Fig. 1: Flow chart of the study

Dentin slabs preparation and selection

Bovine incisors were provided by a company (SIF 1758, Mondelli Indústria de Alimentos S.A., Bauru, SP, Brazil) after animals being slaughtered for consumption. Teeth were selected and cleaned with scaler and water/pumice slurry in dental prophylactic cups. They were then examined by stereomicroscope (Leica S6 D Stereozoom, Mycrosystems Leica AG, Switzerland) with an increase of 40X. The slabs with cracks or structural anomalies were discarded. The roots were separated from their crowns in the cement enamel junction using a low speed water-cooled diamond saw in a sectioning machine (Isomet 1000; Buehler, Lake Bluff, IL, USA). Dentin slabs (3 x 3 x 2 mm) were cut from the cervical third of the root surface. The slabs were fixed in PVC cylinders ¾ inches with polyester resin and were flattened (Politriz DP-9U2, Struers A/S, Copenhagen, DK-2610, Dinamarca) to 2mm with 600 and 1200-grit silicon carbide paper (Hermes Abrasives Ltd., Virginia Beach, VA, USA). Afterwards, specimens were polished with 0.3 µm alumina suspension (Buehler, Lake Bluff, IL, USA) and immersed in the ultrasonic containing deionized water for 10 minutes to clear the surface.

Microhardness readings were performed in microhardness tester (HMV-2000, Shimadzu Corporation, Kyoto, Japão), with the aid of a diamond indenter for Knoop microhardness (KHN), with 10 g static load applied for 20 seconds. A distance of 500 µm was measured from the top edge of the fragment and a lateral distance of 500 µm was measured between each of the indentations. Five microhardness measurments were performed on the dentin surface. Slabs with microhardness values 10% above or 10% below the mean were excluded. The average measurements were considered the outcome value for each slab.

Erosive challenges

For the initial erosive challenges, specimens were submitted to 4 cycles of immersion in orange juice (Fazenda Bela Vista, Água Branca, SP, Brazil), twice a day, for 2 days. Each cycle consisting of the immersion in 20 mL of orange juice (pH = 3.84; Fazenda Bela Vista, Tapiratiba, SP, Brazil) for 5 min under shake (CT155, Cientec, Piracicaba, SP, Brazil). After the erosive challenge, the slabs were rinsed for 10 s and stored in artificial saliva at 37ºC, for four hours. After treatment, further erosive challenges were performed as described above. Prior to the next cycle, slabs were stored in artificial saliva at 37ºC overnight.

Artificial saliva was used as described by McKnight-Hanes and Whitford[31] and modified by Amaechi et al,[32] consisting of: 2.0 g of methyl p-hydroxybenzoate, 10.0 g of sodium carboxymethylcellulose, 0.625 g KCl, 0.059 g $MgCl_2.6H_2O$, 0.166 g $CaCl_2.2H_2O$, 0.804 g K_2HPO_4 and 0.326g KH_2PO_4 (pH 6.75) in 1000 mL of water.

Surface treatment

Dentin slabs were assigned into 4 groups, according to the type of fluoride used prior to Er:YAG laser irradiation: placebo gel-control (pH 5.3) (Bioquanti, Ribeirão Preto, SP, Brazil); titanium tetrafluoride gel (pH 1.3) (Bioquanti, Ribeirão Preto, SP, Brazil) and sodium fluoride gel (pH 5.5) (Bioquanti, Ribeirão Preto, SP, Brazil).

Each fluoride gel was applied with a micro brush on the dentin surfaces (Dentsply Ind. Com. Ltda, Rio de Janeiro, RJ, Brazil). Slabs were exposed to the respective fluoride gel for 1 min, and then removed from the surface with absorbent paper. Control slabs were exposed to a placebo gel, using the same protocol.

An Er:YAG laser device (Kavo Key Laser II; Kavo, Germany), emitting at 60 mJ output and 1 Hz frequency, 0.63 mm (spot size), was used to irradiate the experimental groups. The laser beam was delivered on non-contact and unfocused mode (at a 4- mm distance). The irradiation distance was standardized by using an automatic custom designed apparatus consisting of two parts: a holder to fix the laser handpiece in such a way that the laser beam was delivered perpendicular to the specimen surface, at a constant working distance from the target site; and a semi-adjustable base, on which the fragment was fixed. The semi-adjustable base was automatically moved in both right-to-left and forward-to-back directions, thus allowing the laser beam to provide an accurate and standardized irradiation of the entire dentin sites. The irradiation distance was checked with a ruler for every sample. The spray of water/air (1.5 mL/min) was activated and the regulation of the water flow to cool the dental tissue was adjusted through a valve at the top of the pen (laser hand piece 2051) connected to the equipment through an optical fiber.

Microhardness analysis

The measurements were performed after: 1) erosion-like lesion formation, 2) treatment and 3) subsequent erosive challenges. The protocol used was performed as described above.

Statistical Analysis

For the statistical analysis, the mean of five KHN readings was used for each specimen. After checking normal distribution of data (Kolmogorov-Smirnov test), Knoop microhardness means were submitted to analysis of variance (ANOVA), followed by DUNCAN post-test. A significance level of 5% was adopted. The analysis was performed using Statistical Analysis Software (SAS Institute Inc., USA).

RESULTS AND DISCUSSION

Microhardness values of dentin surfaces increased after the treatments with Placebo gel + Er:YAG laser, TiF_4 + Er:YAG and NaF + Er:YAG laser (p=0.02). After the subsequent erosive challenge, TiF_4 + Er:YAG laser and NaF + Er:YAG laser showed an increase in the microhardness compared to the baseline (after erosion-like lesion formation) (p=0.001), unlike the groups Placebo gel + Er:YAG laser (p=0.664).

Both groups, TiF4 + Er:YAG laser (p=0.012) and NaF + Er:YAG (p=0.002) laser, behaved similarly showing an increase of microhardness after treatment and no difference after subsequent erosive challenges (p=0.705). The control group, Placebo gel + Er:YAG laser, showed microhardness values increased after treatment (p=0.013), however, after subsequent erosive challenges these values decreased (p=0.013).

Table 1 shows the microhardness values after erosion-like lesion formation, treatment and subsequent erosive challenges.

Table 1: Mean (standard deviations) of microhardness values (KHN) of dentin slabs submitted to different treatments.

Treatment	After erosion-like lesion formation (baseline)	After treatment	After subsequent erosive challenges
Placebo + Er:YAG laser	105.4 (4.6) a	110.8 (3.7) b	106.2 (3.9) a
TiF_4 + Er:YAG laser	114.9 (3.9) a	120.5 (3.7) b	121.1 (3.7) b
NaF + Er:YAG laser	105.9 (4.6) a	110.8 (3.4) b	110.7 (3.4) b

Means with the same letter do not differ in by DUNCAN test at a 5% level of significance.

This study has associated the use of a Placebo gel, NaF and TiF_4 combined with the Er:YAG laser application. After subsequent erosive challenges, the slabs treated with TiF_4 + Er:YAG laser and NaF + Er:YAG laser showed a significant increase of microhardness values, different of the slabs treated with Placebo gel + Er:YAG laser that showed the similar average microhardness values compared to the initial condition (after erosion-like lesion formation). Thus, the null hypothesis tested in this study was rejected because the treatment with NaF or TiF_4 combined with Er: YAG laser had a significantly different effect on the microhardness of the eroded dentin.

A previous study demonstrated that a fluoride varnish combined with Er:YAG laser reduced the permeability of eroded dentin. In addition, SEM analysis showed dentinal tubules partially or completely obliterated, which may be considered an additional benefit in controlling the dentin demineralization. [30] This findings are in accordance with a previous study which showed that dentinal tubules were occluded and depressed into craters after Er:YAG laser irradiation, but more marked occlusions were observed when laser and NaF gel were combined. [33]

A hypothesis for the action of fluoride in the dentin may be due to the effect of a precipitation type material CaF_2,[34] which may be retained by the tissue,[35] resulting in a layer that blocks dentinal tubules, as showed by previous studies.[33, 30]

In the case of TiF_4, it reacts with apatite, by dissolution and precipitation, due to its low pH, leading to the formation of a layer rich in calcium fluoride, titanium dioxide and titanium hydrogen phosphate hydrate.[36, 37] This layer provides a dense coating, capable of reducing the permeability of dentin in care of acid attacks.[38] In addition, after application of TiF_4, the nanohardness and reduced modulus of elasticity of the dentin surface were greatly increased.[5]

Studies have been demonstrated that both TiF_4 and NaF are effective in reducing erosion[4] and dentin wear progression under erosive[6,18] and abrasive conditions.[18] However, the single use of TiF_4 (as a solution) was not effective to reduce dentin loss; it was speculated that the CaF2-layer formed might have not been resistant to acidic challenges compared to varnishes.[8]

With the purpose of improving fluoride uptake in dentin, sub-ablative laser irradiation was used on the current study based on previous studies that evaluated Er:YAG laser combined with another fluoride compound.[28, 29]

Analyzing groups independently, after dentin surface treatment, the microhardness values of the Placebo + Er: YAG laser group increased compared to the analysis performed after initial erosion. Laser irradiation, regardless of association with a fluoride compound, was able to increase the microhardness of dentin previously eroded.

In the current study, subablative parameters were used aiming to modify the dentin surface and increase its resistance against erosive challenges. Er:YAG laser modifies dentin providing water loss, changes in structure and composition of collagen, an increase of hydroxyl radicals,[39, 40] and a decreased Ca/P ratio.[40] Er:YAG laser can be used to control the permeability of eroded root dentin, regardless of fluoride varnish application.[41] He et al. found that Er:YAG laser irradiation affected the acid resistance of sub-surface dentin promoting an irregular form of scales or flakes.[26] Contradictorily, after evaluating the amount of mineral dissolved after laser irradiation, it was found that dentin has little or no acid resistance; however, laser irradiation was performed preventively, before the erosive challenge.[42]

Microhardness determination can provide indirect evidence of mineral loss or gain on dental surfaces. The effect of a specific treatment on dental surfaces could be evaluated by this method since it is sensitive to surface changes of the tooth structure.[43] However, there is a lack of studies that evaluated the microhardness of eroded dentin after Er:YAG laser treatment.

Therefore, given the difficulties in controlling the progression of erosive lesions in clinical situations, new strategies that may control mineral loss are of great value. Further studies are required to elucidate the effect of TiF_4 and NaF combined with Er:YAG laser on dentin. In situ studies could be an appropriate choice since this model reproduces a condition that more closely resembles the clinical situation.

CONCLUSION

It can be concluded that the use of TiF_4 and NaF combined with the Er: YAG laser may be an alternative to control the demineralization of eroded dentin.

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CONFLICT OF INTEREST

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service and/or company that is presented in this article.

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