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Effect of Fluoride Varnish Combined with Er:YAG Laser on the Permeability of Eroded Dentin: An *In Situ* Study

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This study evaluated the combined effect of fluoride varnish and Er:YAG laser on the permeability of eroded bovine root dentin. After initial erosive challenge followed by a remineralization period, the specimens were divided in two groups according to the treatment - fluoride varnish and non-fluoride varnish - and were subdivided according to the irradiation protocol: Er:YAG laser (100 mJ, 3 Hz, 12.8 J/cm^2 *per* pulse, non-contact and defocus mode) and non-irradiated. After a lead-in period, 7 volunteers wore a palatal device containing 4 specimens that were subjected to erosive challenges. At the first experimental phase, 4 volunteers used specimens treated with fluoride varnish and fluoride varnish+Er:YAG laser and 3 volunteers used specimens treated with non-fluoride varnish and non-fluoride varnish+Er:YAG laser. After a washout period, volunteers were crossed to treatments, characterizing a 2x2 crossover experiment. At the end of the experimental phase, the quantitative response variable was obtained by permeability analysis and the qualitative response by scanning electron microscopy (SEM). Two-way ANOVA and Tukey-Kramer's test revealed that specimens treated with fluoride varnish+Er:YAG laser showed the lowest permeability and a significant difference was found between this group and the others. When varnish (fluoride/non-fluoride) was applied in the absence of Er:YAG laser, higher permeability was found when compared to the laser-treated groups. SEM evaluations showed partially or completely obliterated dentinal tubules when specimens were treated with fluoride varnish+Er:YAG laser. It may be concluded that Er:YAG laser was able to control the permeability of eroded root dentin and the combination with fluoride varnish increased laser action.

Key words: erosion, Er:YAG laser, fluoride varnish, root dentin.

Introduction

Dental erosion consists of irreversible loss of dental tissue due to a chemical process, which is able to remove the enamel and expose the dentinal tubules, without involving microorganisms (1). When erosion affects dentinal tissue, the peritubular dentin is completely dissolved, increasing the permeability of this tissue and the diameter of the tubules (2), while the intertubular dentin presents greater roughness and porosity (2,3). This process can lead to teeth sensitivity against external stimuli (3), justifying the growing number of researchers interested in dental erosion.

Mechanical, chemical, thermal or osmotic stimuli can cause fluid movement inside the dentinal tubules, with consequent dentin hypersensitivity (4). Thus, effective treatments, capable to interfere in dentinal hydrodynamic conduction (5) could reduce the permeability of this tissue, decreasing the painful symptomatology.

In the dental clinic, one of the most common desensitizing methods used is the professional application of fluoride varnish, in order to occlude the dentinal tubules. When fluoride is applied on the teeth, it creates a barrier at the dental surface, by the calcium fluoride (CaF_2) precipitation that blocks the entrance of the dentinal tubules, reducing the permeability and consequently painful symptomatology (6). The application of fluoride varnish is also a therapeutic option to control erosion at the dentinal substrate (7). However, its role in dental erosion is still discussed, since the mineral (CaF_2) deposited can be dissolved by the most acidic beverages (7).

The search for more effective treatments for controlling erosive lesions, which are one of the causes of cervical dentinal hypersensitivity, has stimulated the development of new technologies, such as different type of lasers. Low-power laser is appropriate to promote biomodulatory effects, minimize pain and reduce inflammatory processes (8,9), while high-power lasers could create an acid resistant layer that can occlude dentinal tubules (10,11).

It has previously been demonstrated in clinical trials that desensitization of hypersensitive dentin with an Er:YAG laser was effective (12). The high absorption of the Er:YAG laser emission wavelength in water may result in an evaporation of the dentinal fluid and smear layer (12). Thus, it could be suggested that deposition of insoluble salts or the accumulation of organic elements in the exposed tubules are responsible for blockade and reduction in diameter of dentinal tubules (12).

Taking into account the effects of Er: YAG laser irradiation on dentin tissue and the use of fluoride compounds in permeability, the purpose of this *in situ* study was to evaluate if the combination of fluoride varnish and Er:YAG laser could promote further sealing of the tubules, with consequent reduction of the permeability of dentin eroded.

Material and Methods

Experimental Design

This crossover double-blind *in situ* study was conducted after a 2-day lead-in period, in two phases of 5 days each, with a 15-day wash-out period between them. The factors under evaluation were: varnish treatment at two levels (fluoride and non-fluoride) and laser irradiation at two levels (Er:YAG and non-irradiated). This study was performed in duplicate because both methods cause destruction of the specimens; in the permeability analysis, the specimens were stained and sectioned and in the in scanning electron microscopy (SEM) analysis the specimens were sputter-coated with a fine gold overlay. Twenty-eight (n=7 *per* group) bovine root dentin slabs were used for analysis of permeability (quantitative response variable) and 12 (n=3 *per* group) for SEM analysis (qualitative response), these slabs were randomly assigned into 7 volunteers. Figure 1 shows the flowchart of this experiment.

Selection of Volunteers

This study was approved by the Ethics Committee of the School of Dentistry of Ribeirão Preto, USP, Brazil (Process no. 92.188). The volunteers were informed about the procedures to be followed in the study, its risks and benefits, and signed an informed written consent.

The sample was composed of 7 healthy adult volunteers, 4 females and 3 males, aged 18-38 years, and residents in Ribeirão Preto, SP, Brazil. The exclusion criteria were: evidence of active carious and non-carious lesions, use of any form of medication likely to interfere with salivary secretion, radio or chemotherapy, use of fixed or removable orthodontic appliances, pregnancy or breastfeeding, and general/systemic diseases.

Selection of Teeth

Freshly extracted bovine incisors stored in 0.1% thymol solution at 4 °C were selected. The periodontal tissue was removed with periodontal probes and cleaned with water, pumice and Robinson brushes mounted in a low speed handpiece. After cleaning, teeth were examined with a stereomicroscope (Leica S6 D Stereozoom, Mycrosystems Leica AG, Switzerland) under 40× magnification to discard those with cracks or abnormalities.

Preparation of Root Dentin Slabs

Two fragments (2x2x2 mm) were obtained from cervical third of each root, using a low-speed water-cooled diamond saw (Isomet 1000; Buehler, Lake Bluff, IL, USA). After checking the dimensions with a digital caliper (Mitutoyo America Suzano, SP, Brazil) the layer of cement was removed with 1200-grit carbide paper (Saint-Gobain Abrasives Ltd., Igarassú, PE Brazil), and specimens were coated with two layers of an acid-resistant nail polish (Colorama, São Paulo, SP, Brazil), on the mesial, distal and lingual surfaces, while the buccal surface was exposed to acid challenges. Afterwards, specimens were sterilized with ethylene oxide in the Department of Ethylene Oxide Sterilization of the Hospital of the Medical School of Ribeirão Preto and another analysis on stereomicroscope was performed in order to select 56 root dentin specimens.

Erosion-like Lesion Formation

The samples were individually immersed in 20 mL of citric acid (0.3%, pH 3.2) in an Erlenmeyer flask, which was placed in an orbital shaker (CT155; Cientec, Piracicaba, SP, Brazil), with stirring velocity of 50 rpm for 2 h (14). Thereafter, specimens were rinsed for 10 s with deionized water and stored in 10 mL of artificial saliva at 37 °C during 24 h. The exposure of dentinal tubules was observed in the SEM analysis (Fig. 2).

Surface Treatment

After erosion-like lesion formation, specimens were randomly assigned according to the treatment to be employed: fluoride varnish or non-fluoride varnish and subdivided according to laser irradiation: Er:YAG laser (100 mJ, 3 Hz) and non-irradiated. The composition of each varnish is shown in Table 1.

The application of varnish (fluoride or non-fluoride) was performed with a microbrush (Vigodent AS Indústria e

Table 1. Characterization of fluoride varnish and non-fluoride varnish

Types of varnish	Fluoride concentration	Constituents	Manufacturer
Fluoride (Duraphat®)	22600 ppm	Solvent: ethanol Base: colophonium, mastix, shellac Active ingredient: Sodium fluoride	Colgate-Palmolive, São Paulo, SP, Brazil
Non-fluoride	0 ppm	Solvent: ethanol Base: acrylic polymer, xylitol Active ingredient: No fluoride	Fórmula & Ação, São Paulo, SP, Brazil

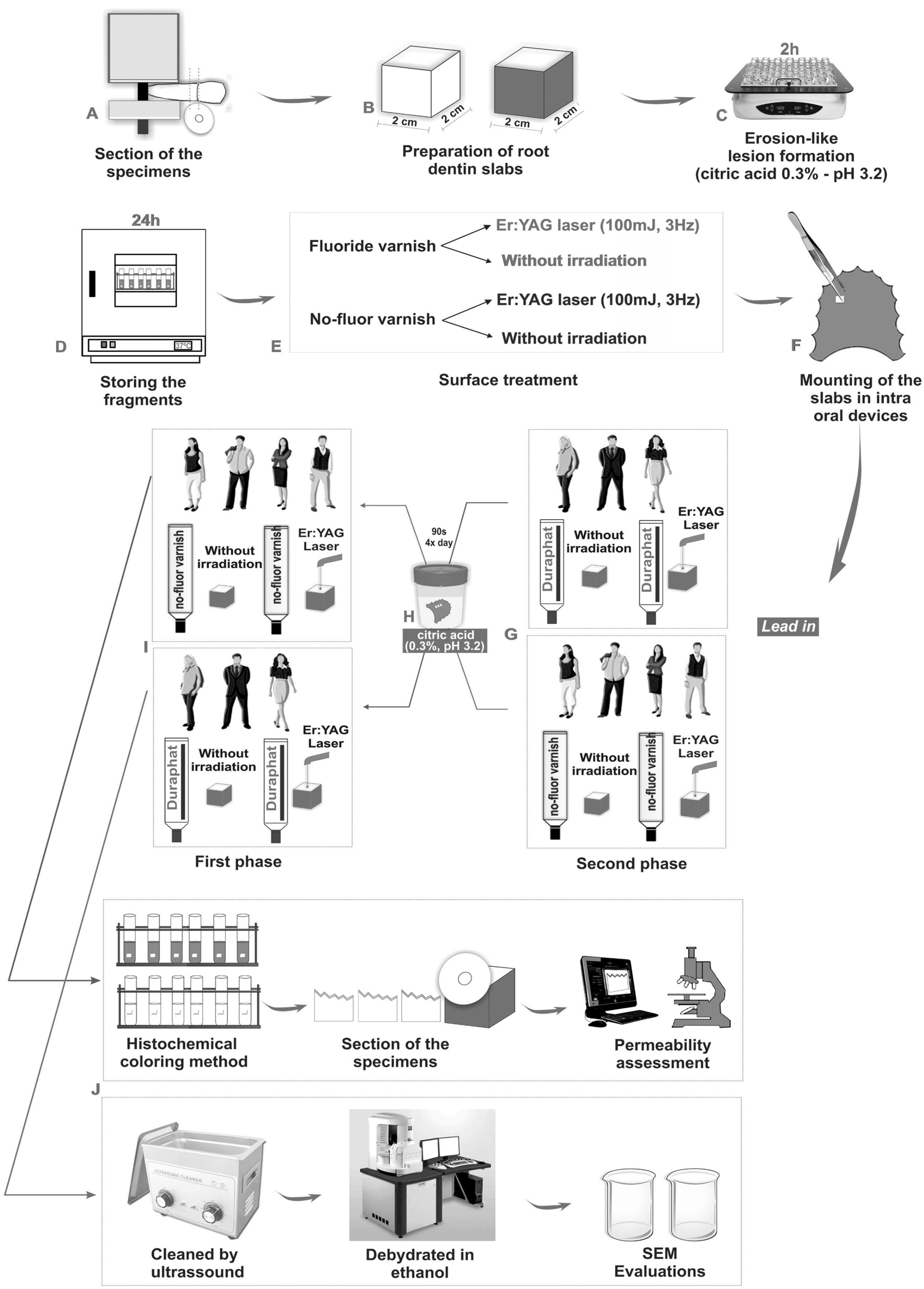

Figure 1. A: Sectioning of specimens. B: Application of nail polish. C: Erosive-like lesion formation. D: Fragments stored in artificial saliva. E: Experimental groups. F: Mounting the specimens in intra oral devices. G: First phase of the experiment. H: Erosive challenges. I: Second phase of the experiment. J: Permeability and SEM analyses.

Comércio, Rio de Janeiro, RJ, Brazil) on the buccal surface of specimens. After 1 min, specimens were individually stored in artificial saliva for 24 h, at 37 °C. Then, varnish layer was carefully removed with scalpel blade #15.

Irradiation with the Er:YAG laser (Twin Light, plus Fotona Fidelis, Ljubljana, Slovenia; 2.94-µm wavelength) was performed with a 100 mJ output, at 3 Hz, (12,14) energy density 12.8 J/cm^2 per pulse, quartz fiber of 835 µm, with a water flow of 1.5 mL/min and was accomplished on non-contact and defocused laser light at a 17 mm distance for 10 s. The irradiation distance was standardized using a custom designed apparatus that promotes the fixation of the laser pen.

Specimens from groups treated only with varnish (fluoride or non-fluoride) were kept in relative humidity at 4 °C during irradiation of the other groups.

Palatal Device Preparation and Mounting of The Slabs

Palatal devices were prepared in acrylic resin, with 2 retention slots (15 x 5 x 3 mm) on either side of the midline, which accommodated dentin slabs. Appliances were checked for their adaptation within the oral cavity and, if necessary, adjustments were made. Dental specimens were fixed with wax and positioned according to a random design.

Intraoral Phases

During a 2-day lead-in period, the volunteers were instructed to use only the toothbrush (Oral- B Indicator 35, Gillette do Brazil Ltda., Manaus, Amazonas, Brazil) and the toothpaste (Colgate® Máxima Proteção Anticáries, Colgate-Palmolive, Osasco, SP, Brazil) supplied by researchers. The volunteers started the *ex vivo* erosive challenges 24 h after the device installation. Volunteers wore an intra-oral appliance with four specimens of dentin. The device was worn from 8 a.m to 5 p.m. While appliances were in place, no food or drink was consumed and oral hygiene could not be performed. All volunteers were instructed toothbrushing immediately after lunchtime and appliances were placed in mouth 1 h later. Biofilm control was prevented by application of one drop of 0.2% chlorhexidine solution in each specimen, in the morning and at the end of each day, during 1 min. Afterwards, palatal device was rinsed with water.

During the first experimental phase, 4 volunteers wore devices containing 2 specimens treated with fluoride varnish and 2 specimens treated with fluoride varnish + Er:YAG laser. The other 3 volunteers used 2 specimens treated with non-fluoride varnish and 2 specimens treated non-fluoride varnish + Er:YAG laser.

Erosive challenges were performed four times daily (9 a.m, 11 a.m, 1 p.m, 3 p.m), *ex vivo*, by immersion of palatal device in 50 mL of 0.3% citric acid (pH 3.2) (13), during 90 s. On completion of first phase, specimens were removed from palatal devices and volunteers had a washout period of 15 days.

After the washout period, new dentin slabs were placed into the palatal appliance and volunteers started the second phase of the current study. Volunteers that wore palatal appliance with 2 specimens from fluoride varnish and 2 specimens from fluoride varnish + Er:YAG laser in the first phase, in the second phase used the palatal device containing 2 slabs from non-fluoride varnish and 2 slabs from non-fluoride varnish + Er:YAG laser and vice-versa.

Histochemical Coloring Method

After the intraoral phases, 28 specimens were individually immersed in 1 mL of 10% copper sulfate aqueous solution (Vetec Química Fina Ltda, Duque de Caxias, RJ, Brasil) for 30 min. Then, specimens were removed from the copper sulfate solution, dried with absorbent paper and immersed in 1 mL of 1% rubianic acid alcoholic solution. Copper ions were revealed by the rubianic acid, resulting in a specific coloration that ranged from dark blue to black, depending on the amount of copper ion penetration. After being stained, specimens were rinsed with distilled water for 15 s, dried and kept individually in a sealed container with cotton moistened in ammonia for 7 days.

Permeability Analysis

Three 400-µm thick slabs were obtained from each sample using a water-cooled diamond saw (Isomet 1000; Buehler, Lake Bluff, IL, USA). Then, slabs were ground on 600- and 1200-grit papers up to the thickness of 200 µm. The images for permeability analysis were obtained with a digital camera attached to the optical microscope (Axiolab Plus, Carl Zeiss, Jena, Germany) (Fig. 3). Permeability was

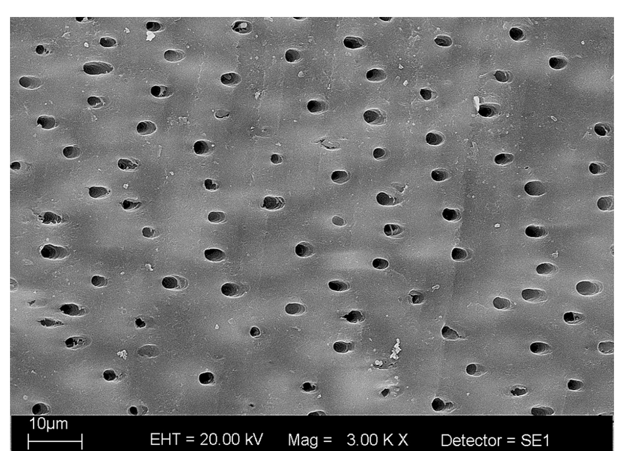

Figure 2. The exposure of dentinal tubules after erosive challenges.

measured as the percentage of copper ions penetrating through the total eroded root dentin thickness using software Axion Vision 3.1 (Kontron Elektronik, Herlev, Denmark). Three measurements of tracer penetration were taken in each one of the three section obtained for each experimental unit. The average among these values represented the permeability in each section. Therefore, it was obtained, for each specimen, nine values of relative permeability, calculated by the ratio between the depth of penetration of cooper ions and total dentin thickness.

SEM Analysis

After erosive challenges, 3 specimens of each group were randomly selected for SEM analysis. The specimens were cleaned by ultrasound (Ultrasonic Cleaner T-1449-D, Odontobrás, Ribeirão Preto, SP, Brazil) for 10 min to remove any residues and immersed into glutaraldehyde solution (2.5%) in sodium cacodylate (0.1 M) buffer with a 7.4 pH (Merck KGaA. Darmstadt, Germany). The samples were dehydrated in increasing ethanol (Labsynth Ltda., Diadema, SP, Brazil) series of 20, 50, 75, 95 and 100% for 20, 20, 30 and 60 min, respectively, and then immersed in hexamethyldisilazane (HDMS - Merck KgaA). The specimens were sputter-coated with a fine gold layer (Bal-Tec, SCD 050 Sputter Coater, Fürstentum, Liechtenstein), examined with a scanning electron microscopy (Zeiss, EVO 50, Cambridge, UK) to obtain and photograph under 1.500× magnification.

Data Analysis

After evaluating the assumptions of normality and homogeneity, data were analyzed using a two-way analysis of variance , at significance level of 5%, and complementary Tukey-Kramer's test to investigate the influence the combination of fluoride varnish to Er:YAG laser on the permeability of eroded root dentin using an *in situ* model. Statistical calculations were performed using SPSS software for Windows, version 12.0 (SPSS, Inc, Chicago, IL, USA). The results of the SEM analysis were not analyzed statistically because the objective of this analysis was to perform a visual and qualitative comparison of the different groups proposed in this study. The SEM analysis was intended to provide a visual and illustrative comparison of the specimens and hence no statistical analysis was performed. The entire ablated surface of each specimen was scanned and the most representative areas were photographed. The SEM images were subjected to a morphological comparison among the groups.

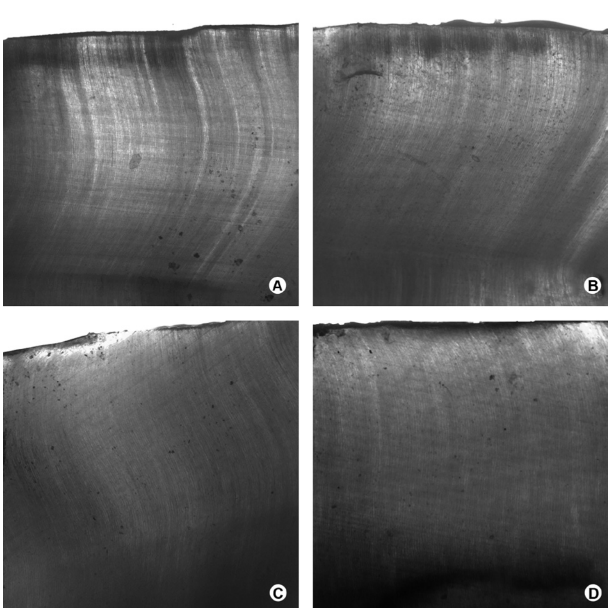

Figure 3. Copper ion penetration into eroded root dentin with different treatments. A: Fluoride varnish, B: Non-fluoride varnish, C: Fluoride varnish + Er:YAG laser D: Non-fluoride varnish + Er:YAG laser.

Results

Permeability Analysis

Two-way ANOVA and Tukey-Kramer's test revealed that specimens treated with fluoride varnish combined with Er:YAG laser ($p=0.001$) showed significantly lower permeability values compared with the other groups.

Er:YAG laser ($p=0.01$) was able to control the permeability of eroded root dentin but the combination of fluoride varnish and Er:YAG laser improved the laser action.

When varnish (fluoride or non-fluoride) was applied without Er:YAG laser ($p=0.01$), higher permeability values were found compared with the irradiated groups. The results of dentin permeability after the challenges are described in Table 2.

SEM Evaluations

Analysis of the control (fluoride varnish and non-fluoride varnish) samples showed dentin with regular and homogeneous appearance (Figs. 4A and 4B). In specimens treated with fluoride varnish (Fig. 4A) was observed after erosive challenges a few open dentinal tubules and most dentinal tubules were partly or completely obliterated.

In Figure 4B (non-fluoride varnish) a higher number of open dentinal tubules was observed.

Table 2. Dentinal permeability after successive erosive challenges to different experimental group (%)

Laser treatment	Fluoride varnish	Non-fluoride varnish
Er:YAG laser	4.88 (±0.52) ^{Bb*}	6.94 (±0.72) ^{Ab*}
Non-irradiated	7.44 (±1.18) ^{Aa*}	8.08 (±0.39) ^{Aa*}

*Uppercase letters indicate similarity between columns and lowercase letters indicate similarity between rows.

The specimens treated with fluoride varnish combined with Er:YAG laser showed dentin with irregular appearance and the dentinal tubules were partially or completely obliterated (Fig. 4C). Non-fluoride varnish combined with Er:YAG showed dentinal tubules partially obliterated or open and few completely obliterated (Fig. 4D).

Discussion

In this *in situ* study, the use of fluoride varnish combined with Er:YAG laser showed lower permeability and a statistically significant difference was found among this group when compared to others. In the SEM analysis, the specimens treated with fluoride varnish combined with Er:YAG laser showed dentin with irregular appearance and the dentinal tubules were partially or completely obliterated. Theses results were expected because when laser is combined with fluoride, it could produce precipitates that seem calcium fluoride deposits on root surfaces (15), and serve as reservoirs to replenish fluoride used during demineralization. Using a NaF gel in combination with Er:YAG laser, Cakar et al. (16) reported that the tubule orifices were occluded and depressed into craters and that the NaF gel layer can provide reactive fluoride ions that would rapidly form salts, resulting in a layer that blocks the openings dentinal tubules. A previous study showed that the depth of fluoride uptake in dentin was significantly higher after laser irradiation (sub-ablative and low-energy) (17). This greater fluoride depth penetration combined with Er:YAG laser may be able to promote an additional benefit in controlling the dentin demineralization.

In the present study, low-energy irradiation was able to control the permeability of eroded root dentin when non-fluoride varnish was combined with Er:YAG laser. High power lasers are able to seal the dentinal tubules or alter the tubule contents by coagulation, protein precipitation, or the creation of insoluble calcium complexes (8,11). This could explain the lower permeability of the groups who received Er:YAG laser irradiation. However, if the use of Er:YAG laser with high energy can promoted irregular surfaces with open dentinal tubules, without smear layer and with protruding tubular aspects (18).

The results of this study revealed that fluoride without laser was not capable of reducing the permeability of dentin. In the early days of erosive challenges, fluoride varnish may has been able to control dental erosion by formation of a calcium fluoride layer, but at the end of intraoral phases this layer may have been lost as observed by Magalhães et al. (7). This may explain the absence of significant difference specimens treated with fluoride varnish (without laser). Other study has found that fluoride (0.2% sodium fluoride mouthwash) was able to control dental erosion, however different to the present study fluoride was applied 4 times a day (1 min each time) during 5 days and samples was placed in a glass of Coca-Cola 4 times a day (2 min each time) during 3 days (19).

Another variable to be considered is the fact that this study was carried out *in situ*, which could interfere with the response of the eroded root dentin to the treatments. The *in situ* model, which represents an intermediate stage between *in vitro* and clinical studies (20), and is appropriate to evaluate the dental erosion since allow the formation of acquired pellicle (21). The acquired pellicle acts like a protection barrier against dental erosion (22), having an inverse relationship between erosion level and pellicle thickness (23).

The citric acid used in this study (0.3%, pH 3.2) was able to cause dentin erosion (13), consequently promote opening of dentinal tubules

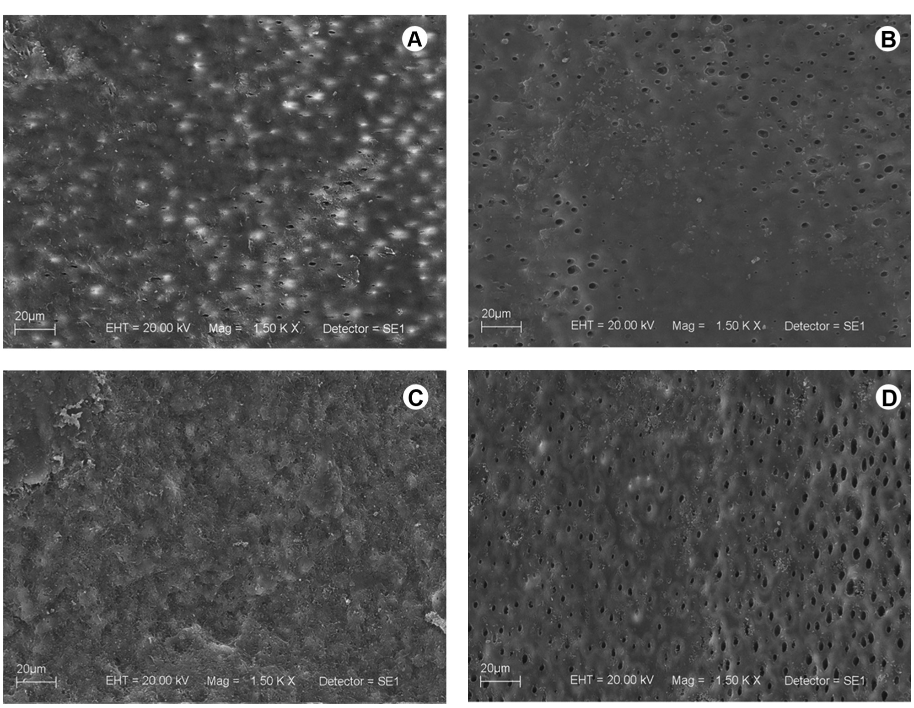

Figure 4. SEM images after intraoral phases. A: Fluoride varnish. B: Non-fluoride varnish. C: Fluoride varnish+ Er:YAG laser. D: Non-fluoride varnish+Er:YAG laser.

and a higher permeability of the dentin (24), and simulating dentin hypersensitivity. This process depends on the contact time with the substrate and the pH of the acid, being observed dissolution of dentin with pH 6, approximately (13). Other authors demonstrated that acidic beverages can promote erosion on root dentin and reduce the phosphorus content after erosive challenge (25).

Erosion can cause an increase in dentin permeability with consequent triggering of clinical dentin hypersensitivity. The present study was based on a clinical previous study (12,14), in which Er:YAG laser parameters employed were effective in reducing hypersensitivity. These parameters are well established by previous studies (12,14) in this study Er:YAG laser irradiation showed additional benefits combined with fluoride varnish.

It may be concluded that Er:YAG laser was able to control the permeability of eroded root dentin and the combination with fluoride varnish increases the laser action.

Resumo

Esse estudo avaliou o feito combinado do verniz fluoretado ao laser de Er:YAG na permeabilidade da dentina radicular erodida. Fragmentos de dentina radicular bovina, foram submetidos a um desafio erosivo inicial com ácido cítrico, que foi seguido por um período de remineralização. Posteriormente, os espécimes foram divididos em relação aos tratamentos: verniz (fluoretado e não fluoretado) e subdivididos de acordo com a irradiação: laser de Er:YAG (100 mJ, 3 Hz, 12.8 J/cm^2 por pulso, sem contato e modo não focado) e não irradiado. Após um período de *lead in*, 7 voluntários utilizaram um dispositivo palatino contendo 4 espécimes que foram submetidos a desafios erosivos. Na primeira fase do experimento, 4 voluntários utilizaram dispositivos com fragmentos tratados com verniz fluoretado e verniz fluoretado+laser de Er:YAG e 3 voluntários utilizaram espécimes tratados com verniz não fluoretado e verniz não fluoretado+laser de Er:YAG. Depois de um período de *wash-out*, os voluntários foram cruzados em relação aos tratamentos, caracterizando um experimento *cross-over* 2x2. Ao final de cada fase experimental, a variável resposta quantitativa foi obtida através da análise de permeabilidade e a qualitativa por microscopia eletrônica de varredura (MEV). A ANOVA dois critérios e teste de Tukey-Kramer revelou que as amostras tratadas com verniz fluoretado+laser de Er:YAG mostraram menor permeabilidade e uma diferença significativa foi encontrada entre este grupo, quando comparado aos demais grupos. Quando o verniz (fluoretado/não fluoretado) foram aplicados na ausência do laser de Er:YAG maiores valores de permeabilidade foram encontrados quando comparados com os grupos irradiados com o laser. A MEV demostrou túbulos dentinários parcialmente ou completamente ocluídos quando as amostras foram tratadas com verniz fluoretado+laser de Er: YAG. Pode-se concluir que o laser de Er:YAG foi capaz de controlar a permeabilidade da dentina radicular erodida e essa ação é aumentada quando o laser está associado ao verniz fluoretado.

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