Effect of Desensitizing Medications with and without Diode Laser Treatment on Dentin Permeability and Surface Morphology

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Abstract

Objective: This study evaluated the effect of desensitizing agents associated with a laser diode on dentin permeability and surface morphology.

Methods: One hundred four bovine root dentin specimens were randomly divided into four groups and two subgroups (n = 13): G1A = no treatment (control), G1B = diode laser, G2A = Oxagel, G2B = diode laser + Oxagel, G3A = MI Paste™, G3B = diode laser + MI Paste™, G4A = Sensitive Pro-Relief™ in-office, G4B = diode laser + Sensitive Pro-Relief™ in-office. For permeability analysis, 10 specimens from each subgroup were immersed in 1% hydrochloric acid (three times for 20 seconds at intervals of 2 hours) to expose the dentinal tubules. The application of desensitizing agents was performed according to the manufacturer's instructions and irradiation with the laser diode was made for 25 sec (contact mode, 970 nm ± 10 nm, 0.7W CW, 10 Hz). After that, the permeability was measured in digitized images in an optical microscope. The remaining specimens (n = 3) were divided into two areas (control and experimental) and the surface morphology was analyzed using scanning electron microscopy. The permeability values were analyzed using analysis of variance (ANOVA).

Results: For dentin permeability, no statistically significant differences were noted among the treatments. No significant differences were verified on the dentin surface by scanning electron microscopy.

Conclusion: The desensitizing agents alone or associated with a diode laser did not affect the root dentin permeability and the dentin surface morphology.

Key words: Dentin permeability, dentin desensitizing agents, diode lasers, scanning electron microscopy, dentin hypersensitivity

Introduction

Dentin hypersensitivity (DH) is a common clinical condition characterized by a short sharp pain arising from exposed dentin in response to thermal, tactile, osmotic or chemical stimuli (Gürsoy *et al.*, 2012). According to the hydrodynamic theory, painful sensation is the result of exposed dentin that is stimulated by fluid displacement inside the tubules, causing a change in intra-pulpal pressure that stimulates nerve endings at the dentin-pulp interface (Brännström, 1966). Despite several therapies presenting clinically proven results (Femiano *et al.*, 2013; Mehta *et al.*, 2014), currently no single desensitizing agent or technique can be considered ideal or long-lasting for the treatment of hypersensitivity (Lopes and Aranha, 2013).

Potassium oxalate is the most commonly used desensitizing agent in the treatment of dentin hypersensitivity (Sales-Peres *et al.*, 2011). It acts on exposed dentin by occluding dentinal tubules through precipitation of calcium oxalate crystals (Kim *et al.*, 2013; Sharma *et al.*, 2013). Different studies (Camilotti *et al.*, 2012; Calabria *et al.*, 2014) have shown promising results in the treatment of dentin hypersensitivity with the application of Oxagel (3% potassium oxalate monohydratate). Further, this agent promotes depolarization of nerve endings, which may explain the short- and long-term effects (Assis *et al.*, 2011).

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The use of desensitizing toothpastes has also demonstrated positive results for *in vivo* studies, including Pro-Argin technology paste based on arginine-calcium carbonate (Cummins, 2011; Moslemi *et al.*, 2013). The mechanism of action of this material is based on the physical sealing of dentinal tubules by the formation of a calcium and phosphate layer in the surface tissue (Petrou *et al.*, 2009), resulting in a significant decrease in dentin permeability and sensitivity through topical application and after brushing (Docimo *et al.*, 2011; Schiff *et al.*, 2011).

Recently, some studies used CPP-ACP (casein phosphopeptide-amorphous calcium phosphate) for the treatment of dentin hypersensitivity. Recaldent™ (CPP-ACP) has demonstrated a potent and prolonged desensitizing effect when used in cervical dentinal hypersensitivity patients (Mahesuti *et al.*, 2014), and it is indicated for prevention of hypersensitivity during dental bleaching (Borges *et al.*, 2012). The application of this product over areas of the exposed dentin results in blockage of the opened dentinal tubules through deposition of a protein component and the binding of calcium and phosphate ions within those components (Tang and Millar, 2010).

A further treatment that has proven to be effective in the management of dentinal hypersensitivity is the use of a laser, which has been tested with different parameters and energy wavelengths (Bader *et al.*, 2014; Dilsiz *et al.*, 2010; Han *et al.*, 2014). For low-power lasers, the desensitizing effect occurs at the neural level, altering the electrical activity or depolarizing effects of afferent fibers (Sgolastra *et al.*, 2011; Lopes *et al.*, 2015). On the other hand, the high power lasers are used to occlude dentinal tubules due to melting and fusion of dentin (Kim *et al.*, 2013). The 980-nm diode laser has a wavelength absorbed by the dentin which provokes a sufficient increase in temperature to obtain a melting effect and reduce or close the dentinal tubules (Umana *et al.*, 2013).

Because the use of these treatments has achieved satisfactory results for the management of dentin hypersensitivity, a possible synergistic effect in exposed dentin caused by the combination of methods must be studied. Therefore, the objective of this study was to evaluate the effect of desensitizing agents associated with or without a laser diode through the analysis of dentin permeability using optical microscopy (OM) and the evaluation of surface morphology using scanning electron microscopy (SEM).

Materials and methods

Experimental design

The layout of this study was a randomized complete block design with 13 bovine dentin specimens per treatment. The study factor was the surface treatment at four levels [control - no treatment, Oxagel (Kota Industria e Comercio Ltda., São Paulo, Brazil), Sensitive Pro-Relief® in-office (Colgate®, Colgate-Pamolive Industria e Comercio Ltda., SB Campos, Brazil) and MI Paste™ (Recaldent™, GC Corporation, Tokyo, Japan) and the application of a diode laser (SIROLaser 2.2, SIRONA Dental, Bensheim, Germany) at two levels [with or without irradiation]. The response variables were the percentage of dentinal permeability (quantitative) and the surface morphology (qualitative).

Root dentin sample preparation

Freshly extracted bovine incisors were cleaned to remove tissue remnants and stored in a 10% formalin solution (pH = 7.0). The roots were separated from their crowns at the cementoenamel junction using a low speed water-cooled diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA). Dentin slabs were cut from the cervical third of the root and polished using 1200-grit aluminum oxide abrasive papers (Norton Abrasives Ltd., São Paulo-SP, Brazil) in a water-cooled grinding device (Arotec APL-4, Arotec Industry and Commerce, São Paulo, Brazil) for 20 seconds to standardize the removal of cementum. A total of 104 specimens were obtained with a 4 mm width, a length corresponding to the size of the tooth surface and a thickness equivalent to the distance between the dentin surface and the pulp chamber wall.

Ten specimens from each subgroup (n = 10) were evaluated for permeability. A piece of adhesive tape with dimensions of 4 mm x 4 mm was put in the central region of each specimen corresponding to the treatment area. The specimens were then coated with two layers of an acid-resistant nail varnish (Colorama-Maybelline, Procosa Produtos de Beleza Ltda., São Paulo, SP, Brazil) and one layer of wax (blue wax, Kota Industria e Comercio Ltda.), except for their buccal surface, corresponding to 16 mm^2 for the treatment area. The other three specimens of each group (n = 3) were used for scanning electron microscopy (SEM) which were delimited in the same manner as described, leaving a 9 mm^2 window. Specimens were stored at 37 ± 0.5º C in 100% relative humidity.

Desensitizing treatments

The specimens were immersed 3 times in 1% hydrochloric acid for 20 seconds in an orbital shaker (CT155, Cientec, Piracicaba, Brazil), with a 2-hour interval between immersions for exposure of the dentinal tubules. The specimens were stored in artificial saliva at 37°C during intervals. A saliva substitute was prepared according to the formulations described by McKnight-Hanes and Whitford (1992) and as modified by Amaechi *et al.* (1999). The saliva substitute was composed of methyl-p-hydroxybenzoate (2.0 g), sodium carboxymethylcellulose (10.0 g), KCl (0.625 g), $MgCl_2 \cdot 6H_2O$ (0.059g), $CaCl_2 \cdot 2H_2O$ (0.166 g), K_2HPO_4 (0.804 g), and KH_2PO_4 (0.326 g) in 1,000 ml of water.

After the initial acid challenge, the specimens were randomly divided into four groups and two experimental subgroups (n = 13), according to the type of treatment: G1A = no treatment (control), G1B = diode laser, G2A = Oxagel, G2B = diode laser + Oxagel, G3A = MI Paste™, G3B = diode laser + MI Paste™, G4A = Sensitive Pro-Relief™ in-office, G4B = diode laser + Sensitive Pro-Relief™ in-office (*Table 1*). In G1B, G2B, G3B and G4B, the irradiations were manually performed using an aluminum gallium arsenide semiconductor 980-nm diode laser. This equipment emits pulses at a wavelength of 970 nm ± 10 nm. The specimens were irradiated with a 200 μm optical fiber. The energy density was set at 222.82 J/cm^2, and the laser was used in noncontact with 0.7 W CW (continuous wave mode) power (energy of 70 mJ), and 10 Hz frequency for 25 seconds. The irradiation was performed on the delimited area of the specimens.

Table 1. Desensitizing treatments for the different experimental groups.

Groups (subgroup)	No irradiation (A)	Irradiation (B)
G1	No treatment (positive control)	Diode laser
G2	Oxagel	Diode laser + Oxagel
G3	MI Paste™	Diode laser + MI Paste™
G4	Sensitive Pro-Relief™ in-office	Diode laser + Sensitive Pro-Relief™ in-office

For G2, G3 and G4, the desensitizing treatment was performed according to the manufacturer's instructions. In G2, Oxagel was applied with a microbrush (Disposable Applicator Brush Fine KG, KG Sorense, Barueri, Brazil) for 2 minutes with slight excess removed with deionized water spray. In G3, MI Paste™ was applied with a microbrush, with the agent remaining in contact with the surface for 3 minutes with slight excess and then the excess was removed with gauze; the product remained on the surface for 2 minutes and was then removed using deionized water spray. In G4, Sensitive Pro-Relief™ in-office was applied with a rubber cup prophylaxis (KG Sorense) at low speed for 3 seconds and then removed with deionized water spray. The procedure was repeated for each specimen.

For G2B, G3B and G4B, the desensitizing agents were applied immediately after irradiation with the laser. After each treatment, the specimens were stored in artificial saliva for 30 minutes at 37° C and then kept in deionized water throughout the experiment.

Dentin permeability assessment

The specimens were immersed in a 10% aqueous solution of copper sulfate (Merck, Darmstadt, Germany) and then in a 1% alcoholic solution of rubeanic acid (Merck), for 30 minutes each; the first 5-minutes of immersion was performed under vacuum. The specimens were then kept in individual vials with ammonia vapor for 7 days (Pécora *et al.*, 1990). After storage, the specimens were sectioned transversely using a water-cooled diamond saw (Isomet 1000; Buehler, Lake Bluff, IL) and a diamond disk (7015-grit, KG Sorensen, Barueri, Brazil). Two sections from each sample were obtained that were approximately 0.5 mm thick. The sections were flattened and polished by hand using water-grained 600 and 1200-grit sandpaper, until the specimens were between 200-150 μm thick. Then, the specimens were dehydrated in alcohol (Labsynth Ltda. Diadema, Brazil) at concentrations of 70, 80, 96 and 100% for 2 hours in each solution and clarified in xylol for 6 hours. The images for permeability analysis were obtained using a digital camera (AxioCam MRC, Carl Zeiss, Jena, Germany) coupled to an optical microscope (AxiostarPlus, Carl Zeiss, Jena, Germany) at x 50 magnification. Permeability was measured in the digitized images (Axion Vision 4.8.2, Carl Zeiss, Germany) as the area of penetration of the copper ions over the total area of the specimen using previously calibrated software. The average of the two sections was considered as the outcome value for each specimen.

Scanning electron microscopy analysis

Prior to the treatments, the specimens to be evaluated under scanning electron microscopy (SEM) had their vestibular surfaces divided into two areas: control area (left) and experimental area (right). For this, half of the surface was covered with a layer of composite resin without the application of an adhesive system to protect the control area during the treatments. The control area of the specimens in G1 (control) did not receive the initial acid challenge, to demonstrate the difference between the intact dentin surface and the exposed dentinal tubules. After the treatments, the specimens were immersed in a 2.5% glutaraldehyde solution (Sigma-Aldrich, St. Louis, MO) buffered with 0.1 M sodium cacodylate (Merck) to a pH of 7.4 for 12 h at 4°C, followed by rinsing in distilled water. Then, the samples were etched with EDTA for 30 seconds, rinsed with air/water spray and placed in an ultrasonic cleaner (T-1449-D; Odontobrás Indústria e Comércio, Ribeirão Preto, SP, Brazil) using distilled water for 3 periods of 5 minutes each. Dehydration was performed by immersing the samples in 25%, 50%, and 75% ethanol baths for 20 minutes each (Labsynth Produtos para Laboratorio, Diadema, SP, Brazil) 95% ethanol for 30 minutes and 100% ethanol for 60 minutes. The specimens were immersed in hexamethyldisilazane (HMDS; Merck) for 10 min and dried in an exhaustion system (Geraldo-Martins *et al.,* 2012).

The specimens were then fixed on stubs with their treated surfaces facing upwards. The specimens were covered with a layer of gold-palladium using a sputtering device (SDC 050, Bal-Tec AG, FL9496, Balzers, Liechtenstein) and examined in a scanning electron microscope (XL30-FEG, JEOL Ltd, Tokyo 190-0012, Japan) operating at 10 – 15 Kv. Photomicrographs were taken at magnifications of 2000 x of the most representative area of each group.

Statistical analysis

For the permeability test, the average of the two sections was considered as the outcome value for each specimen. After homogeneity of variance and normal distribution was confirmed, a two-way analysis of variance (ANOVA) was performed with SPSS 12.0 (SPSS Inc., Chicago, IL, USA) at a significance level of $\alpha = 0.05$. For the SEM, the findings were analyzed using descriptive analyses.

Results

Dentin permeability measurements

In the data analysis, no statistically significant differences in dentin permeability among groups/subgroups were found (*Table 2*).

Table 2. Mean (%) and standard deviation of dentin permeability for the different experimental groups ($\alpha = 0.05$).

Groups (subgroup)	No irradiation (A)	Irradiation (B)
G1	4.94 ± 0.35	4.70 ± 0.54
G2	4.90 ± 0.43	4.86 ± 0.45
G3	4.93 ± 0.35	4.93 ± 0.52
G4	4.54 ± 0.65	4.74 ± 0.42

Morphological analysis (SEM)

In G1A (control), the untreated area represented an intact root dentin tissue that received no treatment. The presence of some tubules with open ends due to initial challenge with hydrochloric acid was noted in G1A. In G1B, there was a small difference between the control area and the area irradiated by the laser, showing a slight decrease in the exposed collagen fibers, with no exposed dentinal tubules in either area. The experimental area in G2 showed a more homogeneous surface with the presence of some crystals deposited on it; the control area presented regions with exposed collagen fibers (asterisks). In G2B, a mesh of collagen fibers could be easily observed in the control area, but a homogeneous surface was observed in the experimental area (*Figure 1*).

In G3A, there was a forming mesh (arrows) in some regions of the experimental dentin surface. In G3B, the control area showed very few exposed fibers and the absence of dentinal tubules, while the experimental area presented small areas of fused dentin (asterisks). In G4A, the control area showed large areas of exposed collagen fibers (arrows), which were smaller in the experimental area. In G4B, there was no difference between regions (control and experimental) and no characteristic structure of dentin tissue was observed in either region (*Figure 2*).

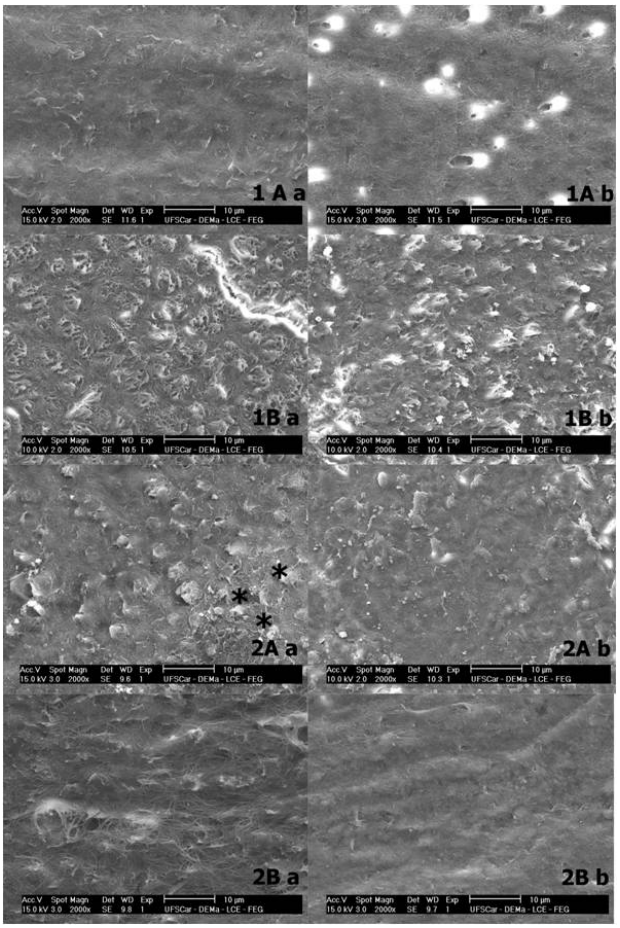

Figure 1. Scanning electron microscopy images representative of groups 1 and 2 (x2000). G1A (control) – a) Control area without acid challenge and treatment: an intact smear layer and no exposed dentinal tubules were observed; b) Control area after acid challenge without treatment: a partially removed smear layer and some exposed dentinal tubules were observed. G1B (diode laser) – a) After acid challenge without treatment; b) After acid challenge and treatment: slight decrease in the exposed collagen fibers with no exposed dentinal tubules in either area. G2A (Oxagel) - a) After acid challenge without treatment: areas with exposed collagen fibers (asterisks); b) After acid challenge and treatment: a more homogeneous surface with the presence of some crystals deposited on it; G2B (diode laser + Oxagel) – a) After acid challenge without treatment: a mesh of collagen fibers could be verified; b) After acid challenge and treatment: a homogeneous surface was observed.

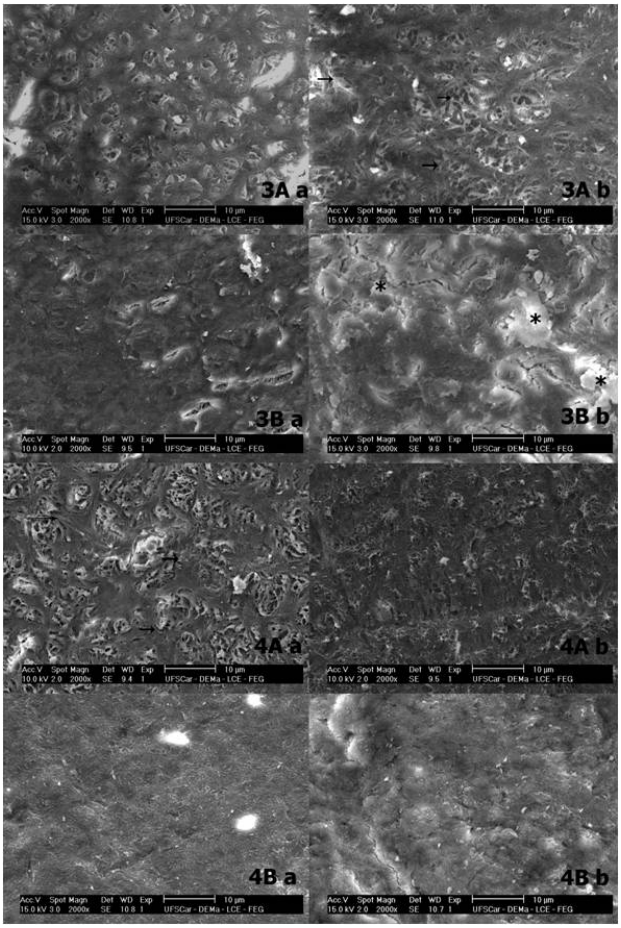

Figure 2. Scanning electron microscopy images representative of G3 and G4 (x2000). G3A (MI Paste™) – a) After acid challenge without treatment: areas with exposed collagen fibers; b) After acid challenge and treatment: there is a forming mesh in some regions (arrows); G3B (diode laser + MI Paste™) – a) After acid challenge without treatment: dentinal tubules absent and very few exposed fibers; b) After acid challenge and treatment: small areas of fused dentin could be observed (asterisks); G4A (Sensitive Pro-Relief™ in-office) - a) After acid challenge without treatment: large areas of exposed collagen fibers (arrows); b) After acid challenge and treatment: decrease of exposed collagen fibers; G4B (diode laser + Sensitive Pro-Relief™ in-office) – There was no difference between control (a) and experimental areas (b) and no characteristic structure of dentin tissue was observed in either.

Discussion

Dentinal hypersensitivity is a complex sensory phenomenon and there is no consensus about the most satisfactory product or technique for treatment (Lopes and Aranha, 2013). In most cases, desensitizing treatments act primarily through mechanical occlusion of the dentinal tubules. However, it has been noted that the results obtained by mechanical occlusion are divergent regarding the effectiveness.

The literature demonstrates that there is a direct relationship between open dentin tubules, permeability and dentin hypersensitivity (Pinto *et al.*, 2010; Sauro *et al.*, 2010). In other words, greater permeability leads to greater clinical hypersensitivity. A greater number of open and wider tubules lead to increased fluid permeability through dentin, which increases stimulus transmission and, eventually, the pain response (Mantzourani and Sharma, 2013). Thus, one of the ways to evaluate the effects of desensitizer treatment is by dentin permeability. The histochemical method used in this study was based on a study by Pécora *et al.* (1990) because of the high reproducibility of their method and the molecular size of the copper ions, which is much smaller than organic dye molecules.

Among the different products used as desensitizing agents, potassium oxalate has demonstrated good results and is the gold standard for the treatment of DH (Sales-Peres *et al.*, 2011; Calabria *et al.*, 2014). In the present study, the Oxagel desensitizing agent did not cause any changes in dentin permeability when used alone or with the laser. According to some authors (Cunha-Cruz *et al.* 2010; Balevi, 2012), with the exception of 3% potassium oxalate monohydratate, the effectiveness of treatments based on other formulations is not yet established, since similar results have been shown in placebo groups. An *in vivo* study for the treatment of hypersensitivity examined the use of a diode laser and Oxagel, immediately and three months after the application (Vieira *et al.*, 2009). That study concluded that both were effective in reducing symptoms. However, longitudinal clinical studies should be performed, as the same results were found when using a placebo gel. According to Pereira *et al.* (2002), because the layer of calcium phosphate is depleted at the dentin surface, potassium oxalate reacts with remaining minerals and precipitates below the surface, which obliterates the tubules. This fact may probably explain the observations in the present study, as an initial erosive challenge was performed prior to application of the agent, therefore decreasing the availability of calcium on the surface, which probably impaired the precipitation. Furthermore, it should be noted that the dentin root surface has a higher amount of collagen fibers and a lower proportion of Ca/P when compared to coronal dentin (Pereira *et al.*, 2002), potentially impairing the action of potassium oxalate.

The application of MI Paste™ with or without the diode laser did not alter dentin permeability. While the literature shows that the precipitation of calcium phosphate (CPP) occludes the dentinal tubules approximately 10-15 μm deep to the surface (Suge *et al.,* 1995), this study did not observe total obliteration of dentinal tubules. This fact probably occurred as a result of prior tissue demineralization: the action of the product induced more remineralization than tubule obliteration, as the

Recaldent™ technology contains casein phosphopeptides (CPP), which carry calcium and phosphate ions in the form of amorphous calcium phosphate (ACP). Although calcium phosphate is normally insoluble at a neutral pH, it remains in a non-crystalline state when in the form of CPP (Tang and Millar, 2010). The CPP-ACP has the ability to stabilize calcium phosphate on the tooth surface, thereby maintaining high concentration gradients of calcium and phosphate ions (Pei *et al.*, 2013). The presence of CPP-ACP at the dentin surface promotes remineralization. However, the ability of remineralization in treating sensitivity has not been demonstrated (Tang and Millar, 2010).

The results of this study indicate no differences when evaluating permeability and surface morphology after the use of Sensitive Pro-Relief™ in-office. Scatolin *et al.* (2012) also found no difference between groups when comparing the use of fluoridated toothpaste with the same Pro-Argin™ technology in eroded root dentin. In contrast, a clinical study showed that an arginine home regimen provided the greatest reduction in tactile and air-blast dentin hypersensitivity and faster relief when compared to potassium and negative control regimens (Elias Boneta *et al.*, 2013). However, there are few studies regarding their effectiveness as an in-office desensitizing agent. According to Petrou *et al.* (2009), the efficiency for hypersensitivity treatment is due to the arginine and calcium carbonate, which accelerates the natural mechanisms of occlusion and helps to form a layer rich in calcium and phosphate on the surface and in the dentinal tubules, which seals them.

With regard to the use of a diode laser, the results of this study showed no difference in dentin permeability when compared to other treatments and the control group. Furthermore, only small areas of laser fusion were observed when used in association with MI Paste™. In general, the surface did not suffer apparent morphological changes, which is probably related to the parameters employed. In contrast, the application of a 980 nm diode laser to the root dentin resulted in different changes, which ranged from modifying the smear layer to initial melting, with these effects enhanced with increasing laser power. The diode laser irradiation at 1.5 W resulted in a slightly irregular surface with partially obliterated dentinal tubules, and irradiation at 3.0 W suggested that an initial melting process occurred (Faria *et al.*, 2013). The use of an 810 and 980 nm diode laser at 0.8 - 1 W can lead to dentinal melting and to narrowing of the dentinal tubules, with the root dentin showing melted areas and total occlusion of tubules at 1.6 - 2 W for the 980 nm diode laser. However, irradiation at 2 W provoked some areas of dentinal ablation and destruction. According to those authors, these alterations are more intense when higher parameters are used (Umana *et al.*, 2013).

In the present study, the desensitizing treatments with or without a laser diode did not influence dentin permeability. The explanation for this finding could be that the initial acid challenge decreased the available minerals on the surface. The different desensitizing treatments mainly act on the mineral content of dentin; therefore, its decreased availability affected the performance. With regard to the 980 nm diode laser, the literature shows that alterations occur in dentin with higher parameters (Faria *et al.*, 2013; Umana *et al.*, 2013).

The divergence in the results can also be associated with the research methodology. The evaluation of permeability used in most studies used hydraulic conductance, which is different from the histochemical method used in this study. Thus, more studies are needed to understand the interaction of different desensitizing agents on the root dentin in order to determine the best treatment protocols.

This study showed that no tested desensitizing agent and diode laser were able to reduce dentin permeability and completely obliterate the dentinal tubules. The association of both did not promote the change of morphology or permeability on the root dentin. More studies are needed to understand the interaction of different desensitizing treatments on the substrate and the best treatment protocols.

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