



Effectiveness and acid/tooth brushing resistance of in-office desensitizing treatments—A hydraulic conductance study

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ABSTRACT

Objective: To evaluate dentin permeability and tubule occlusion of in-office desensitizing treatments, and to analyze their resistance to erosive/abrasive challenges.

Design: Ninety-one 1mm-thick dentin discs were immersed in EDTA solution for 5 min. After analyzing the maximum dentin permeability, the specimens were randomly allocated into 7 experimental groups (n = 10): Control (no treatment); Er,Cr:YSGG laser; Nd:YAG laser; Gluma Desensitizer; Duraphat; Pro-Argin toothpaste; Calcium Sodium Phosphosilicate (CSP) paste. The post-treatment permeability was assessed and then the specimens were subjected to a 5-day erosion-abrasion cycling protocol: 4x/day of immersion in citric acid solution (5 min; 0.3%), followed by exposure to clarified human saliva (60 min). After the first and last acid challenges, specimens were brushed for 15 s, with exposure to the toothpaste slurry for total time of 2 min. Dentin permeability was re-measured (post-cycling). Percentage of dentin permeability for each experimental time was calculated in relation to the maximum permeability (%Lp). Data were analyzed with 2-way repeated measures ANOVA and Tukey tests ($\alpha = 0.05$). Surface modifications were analyzed by scanning electron microscopy.

Results: In both experimental time CSP paste and Gluma Desensitizer did not differ from each other ($p = 0.0874$), and were the only groups that presented significantly lower %Lp than the Control ($p = 0.026$ and $p = 0.022$, respectively). After treatment, they were able to reduce dentin permeability in 82% and 72%, respectively. The %Lp post-cycling was higher than post-treatment value for all groups ($p = 0.008$). Dentin permeability increased 21% for CSP paste and 12% for Gluma, but they remained significant different from Control. Deposits on the surface were observed for CSP paste; and for Gluma, tubule diameters were shown to be smaller.

Conclusions: CSP paste and Gluma Desensitizer were the only treatments able to decrease dentin permeability post-treatment and to sustain low permeability post-cycling.

1. Introduction

Dentin hypersensitivity (DH) has become a common condition nowadays. The pain arises from exposed dentin, stimulus-induced, of short duration, and not related to any disease or other dental pathology (Canadian Advisory Board on Dentin, 2003). DH has shown high prevalence among populations worldwide (West, Seong, & Davies, 2014). Many studies have shown a strong association between the presence of DH and tooth wear (O'Toole & Bartlett, 2017; Yoshizaki et al., 2017). Thus, it is important for dental professionals to investigate the conditions related to tooth wear, with the purpose of eliminating the

predisposing factors.

As DH occurs when dentin with opened dentin tubules has been exposed, treatments capable of causing tubule occlusion and/or acting directly on the pulpal nerves, preventing depolarization and the transmission of the pain to the central system have been suggested (Lin et al., 2013; West, Seong, & Davies, 2015).

The treatments can be performed in-office, where one application usually results in immediate pain relief. These treatments are indicated mainly in cases of localized or severe DH and/or in cases of persistent pain after primary treatment (Canadian Advisory Board on Dentin, 2003; West et al., 2014, 2015). Among the in-office therapies for DH,

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application of fluoride varnish is widely used. The varnishes contain high concentrations of fluoride, capable of forming a mechanical barrier on the exposed dentin (West et al., 2014). Lasers have also been used to treat DH. High power lasers, such as the Nd:YAG and the Er,Cr:YSGG, act through increasing the temperature on the dentin surface, promoting surface changes that would result in tubule occlusion (Naylor, Aranha, Eduardo Cde, Arana-Chavez, & Sobral, 2006; Palazon et al., 2013; Yilmaz, Cengiz, Kurtulmus-Yilmaz, & Leblebicioglu, 2011). Furthermore, they can act on the pulp nerves by diminishing the pain threshold (Ryu et al., 2010; Whitters et al., 1995; Yilmaz, Kurtulmus-Yilmaz, Cengiz, Bayindir, & Aykac, 2011). Some prophylactic pastes may have active agents that act by blocking the dentinal tubules. Examples of these agents are arginine/calcium carbonate and calcium sodium phosphosilicate (CSP) (Earl, Leary, Muller, Langford, & Greenspan, 2011; Kleinberg, 2002; Layer, 2011; Panagakos et al., 2016; Petrou et al., 2009; Wang et al., 2010; Wefel, 2009). Another treatment option is the use of an aqueous solution containing hydroxyethyl methacrylate (HEMA) and glutaraldehyde. These components react with the dentinal fluid, and are polymerized within the dentin tubules (Ishihata, Finger, Kanehira, Shimauchi, & Komatsu, 2011; Qin, Xu, & Zhang, 2006; Schupbach, Lutz, & Finger, 1997).

As regards to the above-mentioned treatments, in addition to acting immediately after their application, they should provide long-term effects. Frequent exposure to acids, together with toothbrushing abrasion, has been shown to play an important role not only in the onset of new lesions, but also, in maintaining the dentin tubules opened (Absi, Addy, & Adams, 1992; Prati, Montebugnoli, Suppa, Valdre, & Mongiorgi, 2003; West et al., 2014). Exposure to acids and abrasion can also influence the durability of treatments (Naylor et al., 2006). Many studies have compared the efficacy of the different in-office desensitizing treatments in vitro and clinically (Ding, Yao, Wang, & Song, 2014; Olley et al., 2012; Palazon et al., 2013; Wang et al., 2010; West et al., 2015; Yang, Wang, Lu, Li, & Zhou, 2016; Yilmaz, Cengiz et al., 2011; Zhu et al., 2015). However, analysis of their resistance to erosive and abrasive challenges has not yet been fully investigated.

In view of the foregoing, the aim of this study was to evaluate dentin permeability and tubule occlusion after the application of several different in-office desensitizing treatments, and to analyze their resistance to erosive and abrasive challenges. The null hypotheses tested were: 1) treatments would not differ regarding their ability to reduce dentin permeability immediately after their application; 2) there would be no difference among treatments relative to their ability to resist the 5-days of erosive-abrasive challenges.

2. Materials and methods

2.1. Specimen preparation

This study was conducted after approval from the local Ethics Committee on Research with Human Beings (process number: 1.402.193). Ninety-one sound human molars were selected. The crowns were first sectioned from the roots, and then dentin discs were prepared from the crowns using a precision cutting machine (Isomet 1000, Buehler Ltd, Lake Buff, Illinois, USA). Two cuts perpendicular to the long axis of the tooth were made in the middle region of the crown, with a distance of approximately 1.5 mm between them, thereby removing the pulp horns and the occlusal enamel. The surfaces were then flattened with a #600 grit abrasive disc in a polishing machine (Buehler Ltd, Lake Buff, Illinois, USA), under constant water cooling, until the discs reached a thickness of 1 mm. This procedure also removed any occlusal enamel that could have remained after the cut. The thickness of the discs was checked with a digital caliper (Mitutoyo, Tokyo, Japan). After the polishing procedure, the specimens were sonicated with distilled water for 3 min, to remove the debris.

2.2. Saliva collection

Human stimulated whole saliva was collected as described earlier (Scaramucci et al., 2016). Before the collection, the volunteers were informed about the nature of the research and had to sign a written informed consent term, in accordance with the regulation of the local ethics committee. The volunteers had to stimulate salivation by chewing a parafilm for 30 s and then swallowing all the saliva produced. After this, they continued chewing the parafilm for 10 min and the saliva produced was collected in an ice-chilled container (Navazesh & Christensen, 1982). The collections were always carried out in the mornings, and the volunteers were instructed not to eat or drink at least 1 h before the collections.

After the collection of each day, saliva from the different volunteers was pooled and immediately centrifuged (20 min, 4 °C, 3226 g-force). The supernatant was separated from the pellet and stored in tubes in a freezer at –80 °C. On the day before use, the necessary amount of saliva was transferred from the freezer to the refrigerator (4 °C). On the day of use, the saliva was removed from the refrigerator and left at room temperature for at least 2 h.

2.3. Maximum dentin permeability

To simulate a hypersensitive dentin, the specimens were immersed in 17% EDTA solution (pH 7.4) for 5 min, in order to open the dentinal tubules. The specimens were rinsed with distilled water and stored in a humid environment until the maximum dentin permeability evaluation, which was considered 100%. The specimens were then randomly allocated into the different experimental groups (n = 10; Table 1).

2.4. Application of the treatments

After opening the dentinal tubules and the maximum permeability assessment, the specimens were immersed in a protein solution for 5 min. The excess solution was removed with absorbent paper, without scrubbing. The protein solution that simulated the dentin fluid was prepared immediately before use by mixing 1 part of fetal bovine serum (FBS; Laborclin) in 4 parts of phosphate buffered saline (pH = 7; Biosystems) (Jain, Reinhardt, & Krell, 2000). This solution was important for the proper mode of action of the components present in the Gluma® Desensitizer.

The in-office treatments were performed according to the protocols established in the literature or according to the manufacturer's recommendations, as described in Table 1. For the purpose of standardization, the toothpaste and the prophylactic paste were mixed with clarified human saliva in the ratio of 1 part of paste to 3 parts of human saliva. The mixture was applied on the dentin surface with a rubber cup for 15 s, then washed with distilled water, and dried carefully with absorbent paper. The specimens were stored in a humid environment and a new evaluation of the dentin permeability was performed (%Lp - post treatment). Three extra specimens per group (total of 21 specimens) were used for the analysis of tubular occlusion and treatment penetration, by scanning electron microscopy (SEM).

2.5. Erosion-abrasion cycle

In order to evaluate the resistance of the desensitizing treatments studied, the treated specimens underwent a 5-day erosion-abrasion cycling protocol. Each cycle consisted of immersion in citric acid (0.3%, natural pH, ~2.6) for 2 min, followed by 60 min of immersion in clarified human saliva, under constant agitation (35 rpm, orbital shaker, AI9000IB, BrILabs), 4 times a day. Thirty min after the first and the last acid immersions, the specimens were brushed in an automatic brushing machine for 15 s (45 cycles, each cycle being considered a back and forth brush movement, load 2N). Brushing was performed with standard brushes and a slurry of toothpaste with clarified human

Table 1
In-office desensitizing treatments: description of the manufacturer, characteristics and protocols.

Group	Treatment	Manufacturer	Characteristics	Protocol
Control	Negative control	–	–	No treatment
Er,Cr:YSGG laser	Er,Cr:YSGG laser	Waterlase, Biolase Technology, Irvine, CA, USA	Wavelength of 2.78 μm, MZ6 tip (600 μm diameter), pulse width of 140–200 μs.	0.25 W; 20 Hz; 0% water; 10% air; 2 irradiations (vertical and horizontal), 10 s each
Nd:YAG laser	Nd:YAG laser	Power Laser ST6, Lares Research, Chico, CA, USA	Wavelength of 1064 nm, pulse width of 120 μs, quartz fiber of 400 μm.	0.6 W; 60 mJ; 10 Hz; ~50 J/cm ² ; 4 irradiations (2 vertical and 2 horizontal), 10 s each
Gluma	Gluma [®] Desensitizer	Heraeus, Hanau, Germany	5% glutaraldehyde, 35% HEMA (2-hydroxyethyl methacrylate).	Application by gently rubbing with microbrush. After 60 s dry with air until loss of luster.
Duraphat	Duraphat [®]	Colgate Palmolive Indústria Comércio, Osasco, Brazil	5% sodium fluoride (22,600 ppm of fluoride)	Application with microbrush.
Pro-Argin toothpaste	Colgate Sensitive Pro-Álvio [™]	Colgate Palmolive Indústria Comércio, Osasco, SP, Brazil	8% arginine, hydrated silica, calcium carbonate, glycerin, water, bicarbonate, sodium saccharine.	Application of the toothpaste mixture (1 part of toothpaste mixed with 3 parts of human saliva) with rubber cup for 15 s.
CSP paste	NUPRO [®] NUSolution Propy	Dentsply Corp., London, United Kingdom	Bioactive glass, NovaMin [®] (15% calcium sodium phosphosilicate), hydrated silica, glycerin, water, bicarbonate, sodium saccharine.	Application of the prophylactic paste mixture (1 part of prophylactic paste mixed with 3 parts of human saliva) with rubber cup for 15 s.

saliva, in the ratio of 1 part of toothpaste to 3 parts of saliva (w/w). A fluoridated toothpaste (Colgate Maximum Caries Protection, Colgate Palmolive, Brazil, sodium monofluorophosphate, 1450 ppm F⁻) was used, which contained no active ingredients specifically for the treatment of dentin hypersensitivity. The total time of exposure to the suspension was 2 min (Scaramucci, Borges, Lippert, Frank, & Hara, 2013). The specimens were rinsed with deionized water and gently dried with absorbent paper after every erosive and abrasive challenge. All experimental procedures were performed at room temperature. After the last abrasion of each day, the specimens were stored in clarified human saliva, under constant agitation, until the beginning of the next cycle. Citric acid solutions were replaced after each demineralization episode (4 times per day) and human saliva was exchanged before the start of each cycle (once daily). After the 5 days of erosion-abrasion cycling, the final dentin permeability was analyzed (%Lp - post cycling).

2.6. Dentin permeability analysis

For the dentin permeability analysis, the specimens were placed in the chamber of a machine for this purpose (Odeme Equipamentos Médicos e Odontológicos Ltda, Luzerna, Brazil), with the occlusal surface facing upward, allowing the water in the system to pass through the disc, from the pulp surface to the occlusal surface, simulating an intrapulpal pressure.

The system was maintained under a constant pressure of 10 psi. For each analysis, a new air bubble was inserted into the system and its linear displacement (mm) by the microcapillary (100 μl) was measured for 3 min. This analysis was repeated 3 times for each specimen. The average of the 3 bubble displacement analyses was converted to flow volume (μL min⁻¹), which was transformed into hydraulic conductance (Lp; min⁻¹ cm²cmH₂O⁻¹). Hydraulic conductance takes into account the area of the specimen through which the water passed (area = 0.058 cm²), the pressure in the system and the flow volume. The dentin permeability of each specimen (%Lp) was expressed as a percentage of the initial hydraulic conductance (considered 100% permeability).

2.7. Scanning electron microscopy

To verify the pattern of tubular occlusion that resulted from the treatments, 21 extra specimens were prepared as described before and analyzed with a scanning electron microscope (SEM; FEI, QuantaFEG 650, Czech Republic) post-application of the treatments (n = 3 specimens/group). Three specimens from each group were randomly selected after the analysis of the %Lp - post-cycling, and used for the SEM analyses post-cycling.

The analyses were made in the center of the specimens after covering with platinum, in the same area that was analyzed for dentin permeability. The settings used were 20 kV and 1500x magnification. The resulting micrographs were qualitatively analyzed (Naylor et al., 2006; Palazon et al., 2013).

2.8. Longitudinal scanning electron microscopy

After analyzing the specimen surfaces by means of SEM, the specimens were fractured in the middle to analyze the depth of the treatments inside the dentinal tubules. For this purpose, they were frozen in nitrogen and then sectioned without coming into contact with the analyzed surfaces. The fractured surfaces were covered with platinum and analyzed by SEM.

2.9. Statistical analyses

Data from the %Lp - post-treatments, and %Lp - post-cycling were evaluated by using the Shapiro-Wilk and Brown-Forsythe tests for analysis of normality and homoscedasticity, respectively. As both assumptions were satisfied, data were analyzed with 2-way ANOVA for

repeated measures, and the differences among the groups were evaluated with Tukey test. The level of statistical significance considered was 5%. Statistical analyses were performed with SigmaPlot 13 software (Systat Software Inc., USA).

3. Results

3.1. Dentin permeability

There were significant differences among the levels of the factor groups ($p = 0.017$) and between the levels of the factor experimental time ($p = 0.008$). The interaction between the factors was not significant ($p = 0.21$).

In both experimental times (post-treatment and post-cycling), CSP paste and Gluma presented significantly lower %Lp than the Control ($p = 0.026$ and $p = 0.022$, respectively), with no significant difference between them ($p = 0.0874$). All the other groups did not differ significantly from the Control ($p > 0.05$). Post-treatment, CSP paste and Gluma were able to reduce dentin permeability in 82% and 72%, respectively. The %Lp post-cycling was higher than the post-treatment value ($p = 0.008$). Dentin permeability increased 21% for CSP paste and 12% for Gluma, but they remained significant different from Control. Fig. 1 shows the results obtained and the statistical differences among groups.

3.2. Scanning electron microscopy

The representative micrographs for each group in both experimental time intervals (post-treatment and post-cycling) are shown in Fig. 2. The micrographs in Fig. 3 represent the longitudinal section of the specimens from each group.

Post-treatment, the Control group, which received no treatment, showed a high number of patent-opened dentin tubules, without presence of smear layer. The transverse micrographs showed the same pattern of opened tubules post-treatment and post-cycling; some small deposits could be observed inside the dentin tubules. The surface of the specimens treated with Duraphat was completely covered by an irregular layer of the varnish, with some visible dentin tubules. However, at the transverse micrographs, only small irregular deposits could be observed inside some of the dentinal tubules. No deposits could be seen post-cycling. For Gluma, no change could be observed in the surface, which showed many opened dentinal tubules; however, the diameter of the tubules seemed to be smaller than those of the Control group. In the

transverse micrograph, we could observe a thin layer covering the entrance of the dentinal tubules and penetrating into them.

Post-cycling, the opened dentinal tubules were more visible; however, there was still a layer covering the entrance of the tubules. For the Nd:YAG laser, opened dentinal tubules were observed, but in the path in which the laser went, partially occluded and occluded dentinal tubules could be observed. Areas with a new surface topography could also be observed, suggestive of dentin melting; these also seemed to be a few micrometers inside the tubules. Small irregular deposits inside the tubules could also be observed. Post-cycling, there was almost no melting, but small irregular deposits could still be observed inside the dentinal tubules. Whereas, the Er,Cr:YSGG laser did not show any significant surface changes. Many dentinal tubules could be seen, but there were small deposits inside the tubules that were partially occluded, and in the transverse micrographs small deposits could be seen inside the dentinal tubules and the opened dentin tubules were clearly observed post-treatment. Post-cycling, the deposits were scarce and the dentinal tubules were more widely opened than before.

The CSP paste promoted crystal-like deposits on the dentin surface and most of the tubules were completely or partially occluded by small deposits. These deposits were present at the entrance of the dentinal tubules, occluding them. Post-cycling, this group continued to show deposits inside the tubules, although more opened tubules were observed. The specimens treated with Pro-Argin toothpaste showed small granular deposits on the surface, with many opened dentinal tubules. There were, however, some partially occluded dentin tubules with small deposits within them; these were also observed inside the tubules in the transverse micrograph. These deposits were more irregular after cycling. Opened dentin tubules could be observed in both experimental time intervals for this group.

4. Discussion

According to our results, although all groups were able to reduce dentin permeability after treatments when compared with the maximum permeability condition, only Gluma and CPS paste differed significantly from the Control group. In view of this, our first study hypothesis was rejected. These two groups were also capable of sustaining tubule occlusion after cycling; therefore, our second hypothesis was also rejected.

Among the treatments for DH tested in the present study, Gluma was able to reduce 72% of the dentin permeability. This aqueous solution contains 35% hydroxyethyl methacrylate (HEMA) and 5%

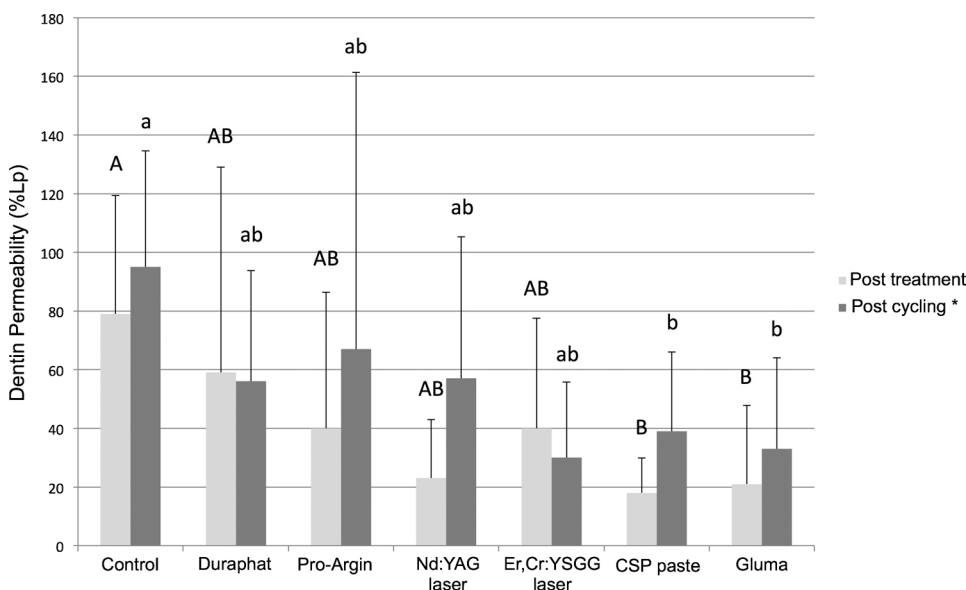


Fig. 1. Mean and standard deviation of the percentage of dentin permeability (%Lp) for each experimental group, in both experimental times (post treatment and post cycling). Different capital letters indicate significant differences among groups post treatment and different lowercase letters indicate significant differences among groups post cycling ($p < 0.05$); * Indicates significant difference between experimental times for all groups ($p < 0.05$).

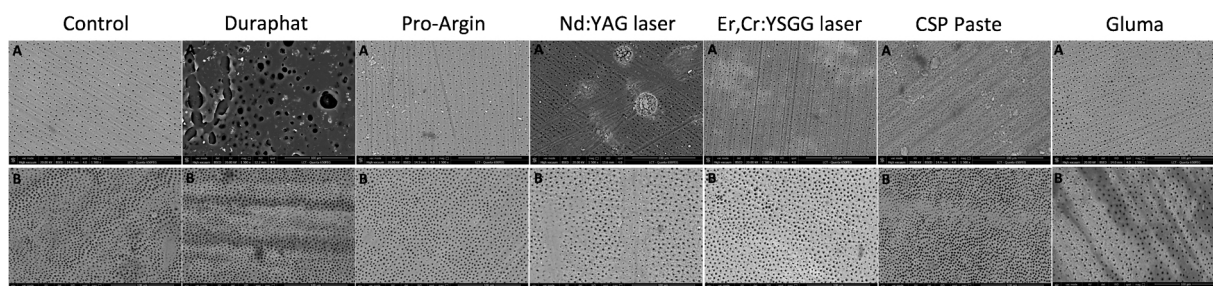


Fig. 2. Representative superficial micrographs for each group. A: Post-treatment; B: Post-cycling.

glutaraldehyde. The reaction of the latter agent with the proteins of the dentinal fluid forms precipitates and acts in the polymerization of HEMA, occluding the dentinal tubules (Qin et al., 2006; Schupbach et al., 1997). A previous study failed to show the efficacy of Gluma in decreasing dentin permeability when using an *in vitro* model with the absence of proteins of the dentin fluid (Kim, Kim, Kim, & Lee, 2013). In view of this, in the present study, before applying the treatments, the specimens were immersed in a protein solution that presented similar characteristics to those of the dentinal fluid.

Although the efficacy of Gluma in occluding the dentinal tubules has also been shown previously (Duran, Sengun, Yildirim, & Ozturk, 2005; Ishihata et al., 2011), the resistance of this agent to erosive challenges has not been fully explored. In the present study, even after the erosive and abrasive challenges, which resulted in 12% higher dentin permeability, Gluma continued to present lower dentin permeability than the Control, demonstrating that it had a certain chemical and mechanical resistance. This result was corroborated by some clinical studies, which have shown a decrease in DH immediately after application of Gluma, and when re-evaluated after some months (Lopes, de Paula Eduardo, & Aranha, 2017; Lopes, Eduardo Cde, & Aranha, 2015; Yu, Liang, Jin, Fu, & Hannig, 2010).

After application, the prophylactic paste containing calcium sodium phosphosilicate (CSP; known as NovaMin[®]) decreased dentin permeability by 82%. The effectiveness of this agent in occluding the dentinal tubules, as well in decreasing the pain of DH has been shown in *in vitro* (Joshi, Gowda, & Joshi, 2013; LaTorre & Greenspan, 2010) and clinical investigations (Litkowski & Greenspan, 2010; Millemann et al., 2012), respectively. CSP reacts with dentin to form a mineralized layer that obliterates the dentinal tubules (Burwell, Litkowski, & Greenspan, 2009). The micrographs from this group showed that the crystal-like deposits on the dentin surface and the small deposits inside the dentinal tubules were responsible for their occlusion. This has also been shown in another study testing a toothpaste containing CSP (Zhong et al., 2015). After cycling, dentin permeability was increased by 21% in this group, but it remained different from the Control. This data suggested that a single application of the CSP paste is sufficient to withstand the erosive and abrasive challenges that may be present in the oral environment on a daily basis. This result was in agreement with other reports that have shown the capacity of CSP to occlude the dentinal tubules and to resist to chemical and mechanical challenges (Cunha,

Garofalo, Scaramucci, Zzell, & Aranha, 2017; West et al., 2011).

Er,Cr:YSGG laser showed no significant difference in dentin permeability from the control post-treatment and post-cycling. Contrary to this result, it was previously shown that this high power laser had a positive effect on the control and treatment of DH (Aranha & Eduardo Cde, 2012; Yilmaz, Cengiz et al., 2011, 2011). The suggestion was that Er,Cr:YSGG laser irradiation, used in the same parameters as those of the present study, could obliterate the dentinal tubules by deposition of insoluble salts within them (Schwarz, Arweiler, Georg, & Reich, 2002; Yilmaz, Cengiz et al., 2011). In the present study, although some deposits inside the dentinal tubules could be observed in the micrographs, this was unable to significantly reduce dentin permeability. This was perhaps because the laser did not homogeneously irradiate the whole dentin surface; thus, there was a great chance that in some specimens, the small area analyzed for dentin permeability was not efficiently modified by the laser, possibly underestimating the effect of the laser.

Similarly to the Er,Cr:YSGG laser, Nd:YAG laser was not able to decrease dentin permeability in both experimental times, in comparison with the Control. An *in vitro* study showed that Nd:YAG laser was able to occlude the dentinal tubules and form a more acid resistant dentin layer, which was able to persist up to 6 h of immersion in acid beverages (Naylor et al., 2006). However, contrary to these findings, another study could not observe an effective maintenance of tubule occlusion with the Nd:YAG laser irradiation after erosive and abrasive challenges (Palazon et al., 2013). In addition, there are evidences showing that Nd:YAG laser may not be effective in preventing the progression of dentin erosion (João-Souza, Scaramucci, Hara, & Aranha, 2015). In the micrographs of this group after treatment, the path through which the laser went could be clearly seen, as the dentinal tubules appeared occluded in these areas. In this experimental time interval, the reason for the lack of effect of this laser could be the same as stated above for Er,Cr:YSGG laser. Post-cycling, the laser-irradiated areas could be observed to be less clear in the micrographs, suggesting that they were removed by the challenges.

The fluoride varnish (Duraphat) and the toothpaste containing arginine/calcium carbonate (Pro-Argin toothpaste) were also unable to reduce dentin permeability in both experimental times. The varnish has a high concentration of sodium fluoride and its desensitizing effect is the result of the formation of layer of CaF₂ on the surface, occluding the dentinal tubules (Calabria et al., 2014; Orchardson & Gillam, 2006).

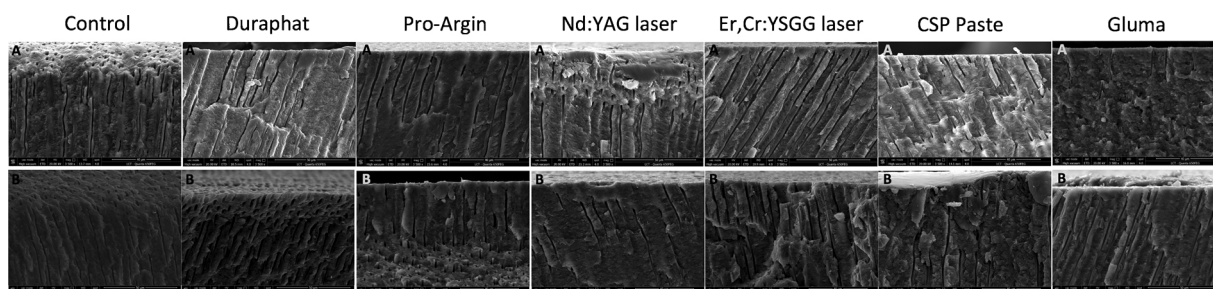


Fig. 3. Representative transverse micrographs for each group. A: Post-treatment; B: Post-cycling.

This occlusion is also a result of the presence of the varnish itself on the dentin surface, blocking the tubules as long as it stays on the tooth (West et al., 2014). However, the micrographs showed an irregular layer of the varnish, which seemed to be porous, explaining the lack of difference in dentin permeability when compared with the Control group. Differently from the results of the present study, another report was able to show an efficacy of fluoride varnish in decreasing dentin permeability, even after exposure for 6 min to 6% citric acid (pH 2.1) (Calabria et al., 2014). The layer of varnish observed post-treatment was completely removed post-cycling. In view of this, it could be suggested that the challenges may have detached the varnish and dissolved its deposits from the surface.

In the present study, the Pro-Argin toothpaste was used as an in-office treatment, as no difference in dentin permeability between the toothpaste and the prophylactic paste containing the same concentration of arginine was observed (Petrou et al., 2009). Although this paste has shown promising in vitro results in dentin occlusion (Patel, Chopra, Vandeven, & Cummins, 2011; Petrou et al., 2009; Schiff et al., 2011), contrary results, which demonstrated that it was unable to promote tubular occlusion, have also been reported (Cunha et al., 2017; Palazon et al., 2013; West et al., 2011), corroborating to our results. It is worth mentioning that the Pro-Argin toothpaste and the CSP paste were mixed with human saliva before application on dentin surface with rubber cup. This was performed to simulate the clinical scenario in which the components will naturally get in contact with saliva forming a slurry.

After cycling, all groups presented lower dentin permeability than the maximum permeability. This may have occurred due to the use of the toothpaste during the abrasive challenges that presented abrasives; thus it may have formed a smear layer on the dentin surface (Palazon et al., 2013). Although almost no smear layer could be clearly observed in the micrographs, there was reduction in the tubular diameter.

One limitation of the present study was the device used to determine the dentin permeability that evaluated only a small area in the center of the specimen (of 0.058 cm²) and not the whole surface of the dentin disc. This could have resulted in the evaluation of an area where there was no treatment present, thus underestimating the results or, on the contrary, overestimating the result when there was a higher deposition of some product. It is important to mention that care was taken to perform the readouts at all times in the same place on the specimen, with each specimen serving as its own control. Moreover, the intrapulpal pressure simulated by the system was constant during all the analyses. In addition, we attempted to apply the treatments as uniformly as possible across the entire dentine surface. However, non-homogeneous characteristics of the surfaces after treatment could be observed in the micrographs. In view of this, care should be taken when extrapolating the results obtained here to the clinical scenario. Nevertheless, evaluating in-office desensitizing treatments in vitro allows the control of undesirable variables, assisting the comparison between groups and the explanation of their possible mode of action.

5. Conclusion

Under the conditions and limitations of this in vitro investigation, it could be concluded that CSP paste and Gluma Desensitizer were the only treatments able to decrease dentin permeability after application and after the erosive-abrasive cycling. Thus, they may be considered effective and resistant treatments for DH.

Declarations of interest

None.

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References

- Absi, E. G., Addy, M., & Adams, D. (1992). Dentine hypersensitivity—the effect of tooth-brushing and dietary compounds on dentine in vitro: An SEM study. *Journal of Oral Rehabilitation*, 19(2), 101–110.
- Aranha, A. C., & Eduardo Cde, P. (2012). Effects of Er:YAG and Er,Cr:YSGG lasers on dentine hypersensitivity. Short-term clinical evaluation. *Lasers in Medical Science*, 27(4), 813–818.
- Burwell, A. K., Litkowski, L. J., & Greenspan, D. C. (2009). Calcium sodium phosphosilicate (NovaMin): Remineralization potential. *Advances in Dental Research*, 21(1), 35–39.
- Calabria, M., Porfirio, R., Fernandes, S., Wang, L., Buzalaf, M., Pereira, J., ... Magalhães, A. (2014). Comparative in vitro effect of TlF4 to NaF and potassium oxalate on reduction of dentin hydraulic conductance. *Operative Dentistry*, 39(4), 427–432.
- Canadian Advisory Board on Dentin, H. (2003). Consensus-based recommendations for the diagnosis and management of dentin hypersensitivity. *Journal Canadian Dental Association*, 69(4), 221–226.
- Cunha, S. R., Garofalo, S. A., Scaramucci, T., Zzell, D. M., & Aranha, A. C. C. (2017). The association between Nd:YAG laser and desensitizing dentifrices for the treatment of dentin hypersensitivity. *Lasers in Medical Science*, 32(4), 873–880.
- Ding, Y. J., Yao, H., Wang, G. H., & Song, H. (2014). A randomized double-blind placebo-controlled study of the efficacy of Clinpro XT varnish and Gluma dentin desensitizer on dentin hypersensitivity. *American Journal of Dentistry*, 27(2), 79–83.
- Duran, I., Sengun, A., Yildirim, T., & Ozturk, B. (2005). In vitro dentine permeability evaluation of HEMA-based (desensitizing) products using split-chamber model following in vivo application in the dog. *Journal of Oral Rehabilitation*, 32(1), 34–38.
- Earl, J. S., Leary, R. K., Muller, K. H., Langford, R. M., & Greenspan, D. C. (2011). Physical and chemical characterization of dentin surface following treatment with NovaMin technology. *Journal of Clinical Dentistry*, 22(3), 62–67.
- Ishihata, H., Finger, W. J., Kanehira, M., Shimauchi, H., & Komatsu, M. (2011). In vitro dentin permeability after application of Gluma(R) desensitizer as aqueous solution or aqueous fumed silica dispersion. *Journal of Applied Oral Science*, 19(2), 147–153.
- Jain, P., Reinhardt, J. W., & Krell, K. V. (2000). Effect of dentin desensitizers and dentin bonding agents on dentin permeability. *American Journal of Dentistry*, 13(1), 21–27.
- João-Souza, S. H., Scaramucci, T., Hara, A. T., & Aranha, A. C. (2015). Effect of Nd:YAG laser irradiation and fluoride application in the progression of dentin erosion in vitro. *Lasers in Medical Science*, 30(9), 2273–2279.
- Joshi, S., Gowda, A. S., & Joshi, C. (2013). Comparative evaluation of NovaMin desensitizer and Gluma desensitizer on dentinal tubule occlusion: A scanning electron microscopic study. *Journal of Periodontal & Implant Science*, 43(6), 269–275.
- Kim, S. Y., Kim, E. J., Kim, D. S., & Lee, I. B. (2013). The evaluation of dentinal tubule occlusion by desensitizing agents: A real-time measurement of dentinal fluid flow rate and scanning electron microscopy. *Operative Dentistry*, 38(4), 419–428.
- Kleinberg, I. (2002). SensiStat. A new saliva-based composition for simple and effective treatment of dentinal sensitivity pain. *Dentistry Today*, 21(12), 42–47.
- LaTorre, G., & Greenspan, D. C. (2010). The role of ionic release from NovaMin (calcium sodium phosphosilicate) in tubule occlusion: An exploratory in vitro study using radio-labeled isotopes. *Journal of Clinical Dentistry*, 21(3), 72–76.
- Layer, T. M. (2011). Development of a fluoridated, daily-use toothpaste containing NovaMin technology for the treatment of dentin hypersensitivity. *Journal of Clinical Dentistry*, 22(3), 59–61.
- Lin, P. Y., Cheng, Y. W., Chu, C. Y., Chien, K. L., Lin, C. P., & Tu, Y. K. (2013). In-office treatment for dentin hypersensitivity: A systematic review and network meta-analysis. *Journal of Clinical Periodontology*, 40(1), 53–64.
- Litkowski, L., & Greenspan, D. C. (2010). A clinical study of the effect of calcium sodium phosphosilicate on dentin hypersensitivity—proof of principle. *Journal of Clinical Dentistry*, 21(3), 77–81.
- Lopes, A. O., de Paula Eduardo, C., & Aranha, A. C. C. (2017). Evaluation of different treatment protocols for dentin hypersensitivity: An 18-month randomized clinical trial. *Lasers in Medical Science*, 32(5), 1023–1030.
- Lopes, A. O., Eduardo Cde, P., & Aranha, A. C. (2015). Clinical evaluation of low-power laser and a desensitizing agent on dentin hypersensitivity. *Lasers in Medical Science*, 30(2), 823–829.
- Milleman, J. L., Milleman, K. R., Clark, C. E., Mongiello, K. A., Simonton, T. C., & Proskin, H. M. (2012). NUPRO sensodyne prophylaxis paste with NovaMin for the treatment of dentin hypersensitivity: A 4-week clinical study. *American Journal of Dentistry*, 25(5), 262–268.
- Navazesh, M., & Christensen, C. M. (1982). A comparison of whole mouth resting and stimulated salivary measurement procedures. *Journal of Dental Research*, 61(10), 1158–1162.
- Naylor, F., Aranha, A. C., Eduardo Cde, P., Arana-Chavez, V. E., & Sobral, M. A. (2006). Micromorphological analysis of dentinal structure after irradiation with Nd:YAG laser and immersion in acidic beverages. *Photomedicine and Laser Surgery*, 24(6), 745–752.
- O'Toole, S., & Bartlett, D. (2017). The relationship between dentine hypersensitivity, dietary acid intake and erosive tooth wear. *Journal of Dentistry*, 67, 84–87.
- Olley, R. C., Pilecki, P., Hughes, N., Jeffery, P., Austin, R. S., Moazzez, R., ... Bartlett, D. (2012). An in situ study investigating dentine tubule occlusion of dentifrices following acid challenge. *Journal of Dentistry*, 40(7), 585–593.
- Orchardson, R., & Gillam, D. G. (2006). Managing dentin hypersensitivity. *Journal of the American Dental Association*, 137(7), 990–998 quiz 1028–1029.
- Palazon, M. T., Scaramucci, T., Aranha, A. C., Prates, R. A., Lachowski, K. M., Hanashiro, F. S., ... Youssef, M. N. (2013). Immediate and short-term effects of in-office desensitizing treatments for dentinal tubule occlusion. *Photomedicine and Laser Surgery*,

- 31(6), 274–282.
- Panagakos, F. S. D. E., Morrison, B. M., Mello, S., Santarpia, R. P., Williams, M., & Zhang, Y. P. (2016). Advanced dentin hypersensitivity technology: A comprehensive review of the benefits of Pro-Argin™ oral care products. *Journal of Clinical Dentistry*, 27(Suppl), S1–S15.
- Patel, R., Chopra, S., Vandeven, M., & Cummins, D. (2011). Comparison of the effects on dentin permeability of two commercially available sensitivity relief dentifrices. *Journal of Clinical Dentistry*, 22(4), 108–112.
- Petrou, I., Heu, R., Stranick, M., Lavender, S., Zaidel, L., Cummins, D., ... Gimzewski, J. K. (2009). A breakthrough therapy for dentin hypersensitivity: How dental products containing 8% arginine and calcium carbonate work to deliver effective relief of sensitive teeth. *Journal of Clinical Dentistry*, 20(1), 23–31.
- Prati, C., Montebugnoli, L., Suppa, P., Valdre, G., & Mongiorgi, R. (2003). Permeability and morphology of dentin after erosion induced by acidic drinks. *Journal of Periodontology*, 74(4), 428–436.
- Qin, C., Xu, J., & Zhang, Y. (2006). Spectroscopic investigation of the function of aqueous 2-hydroxyethylmethacrylate/glutaraldehyde solution as a dentin desensitizer. *European Journal of Oral Sciences*, 114(4), 354–359.
- Ryu, J. J., Yoo, S., Kim, K. Y., Park, J. S., Bang, S., Lee, S. H., ... Hwang, S. W. (2010). Laser modulation of heat and capsaicin receptor TRPV1 leads to thermal anti-nociception. *Journal of Dental Research*, 89(12), 1455–1460.
- Scaramucci, T., Borges, A. B., Lippert, F., Frank, N. E., & Hara, A. T. (2013). Sodium fluoride effect on erosion-abrasion under hyposalivatory simulating conditions. *Archives of Oral Biology*, 58(10), 1457–1463.
- Scaramucci, T., Joao-Souza, S. H., Lippert, F., Eckert, G. J., Aoki, I. V., & Hara, A. T. (2016). Influence of toothbrushing on the antierosive effect of film-forming agents. *Caries Research*, 50(2), 104–110.
- Schiff, T., Mateo, L. R., Delgado, E., Cummins, D., Zhang, Y. P., & DeVizio, W. (2011). Clinical efficacy in reducing dentin hypersensitivity of a dentifrice containing 8.0% arginine, calcium carbonate, and 1450 ppm fluoride compared to a dentifrice containing 8% strontium acetate and 1040 ppm fluoride under consumer usage conditions before and after switch-over. *Journal of Clinical Dentistry*, 22(4), 128–138.
- Schupbach, P., Lutz, F., & Finger, W. J. (1997). Closing of dentinal tubules by Gluma desensitizer. *European Journal of Oral Sciences*, 105(5 Pt 1), 414–421.
- Schwarz, F., Arweiler, N., Georg, T., & Reich, E. (2002). Desensitizing effects of an Er:YAG laser on hypersensitive dentine. *Journal of Clinical Periodontology*, 29(3), 211–215.
- Wang, Z., Sa, Y., Sauro, S., Chen, H., Xing, W., Ma, X., ... Wang, Y. (2010). Effect of desensitizing toothpastes on dentinal tubule occlusion: A dentine permeability measurement and SEM in vitro study. *Journal of Dentistry*, 38(5), 400–410.
- Wefel, J. S. (2009). NovaMin: Likely clinical success. *Advances in Dental Research*, 21(1), 40–43.
- West, N., Seong, J., & Davies, M. (2014). Dentine hypersensitivity. *Monographs in Oral Science*, 25, 108–122.
- West, N. X., Macdonald, E. L., Jones, S. B., Claydon, N. C., Hughes, N., & Jeffery, P. (2011). Randomized in situ clinical study comparing the ability of two new desensitizing toothpaste technologies to occlude patent dentin tubules. *Journal of Clinical Dentistry*, 22(3), 82–89.
- West, N. X., Seong, J., & Davies, M. (2015). Management of dentine hypersensitivity: Efficacy of professionally and self-administered agents. *Journal of Clinical Periodontology*, 42(Suppl 16), S256–302.
- Whitters, C. J., Hall, A., Creanor, S. L., Moseley, H., Gilmour, W. H., Strang, R., ... Orchardson, R. (1995). A clinical study of pulsed Nd:YAG laser-induced pulpal analgesia. *Journal of Dentistry*, 23(3), 145–150.
- Yang, Z. Y., Wang, F., Lu, K., Li, Y. H., & Zhou, Z. (2016). Arginine-containing desensitizing toothpaste for the treatment of dentin hypersensitivity: A meta-analysis. *Clin Cosmet Investig Dent*, 8, 1–14.
- Yilmaz, H. G., Cengiz, E., Kurtulmus-Yilmaz, S., & Leblebicioglu, B. (2011). Effectiveness of Er,Cr:YSGG laser on dentine hypersensitivity: A controlled clinical trial. *Journal of Clinical Periodontology*, 38(4), 341–346.
- Yilmaz, H. G., Kurtulmus-Yilmaz, S., Cengiz, E., Bayindir, H., & Aykac, Y. (2011). Clinical evaluation of Er,Cr:YSGG and GaAlAs laser therapy for treating dentine hypersensitivity: A randomized controlled clinical trial. *Journal of Dentistry*, 39(3), 249–254.
- Yoshizaki, K. T., Francisconi-Dos-Rios, L. F., Sobral, M. A., Aranha, A. C., Mendes, F. M., & Scaramucci, T. (2017). Clinical features and factors associated with non-carious cervical lesions and dentin hypersensitivity. *Journal of Oral Rehabilitation*, 44(2), 112–118.
- Yu, X., Liang, B., Jin, X., Fu, B., & Hannig, M. (2010). Comparative in vivo study on the desensitizing efficacy of dentin desensitizers and one-bottle self-etching adhesives. *Oper Dent*, 35(3), 279–286.
- Zhong, Y., Liu, J., Li, X., Yin, W., He, T., Hu, D., ... Wang, Y. (2015). Effect of a novel bioactive glass-ceramic on dentinal tubule occlusion: An in vitro study. *Australian Dental Journal*, 60(1), 96–103.
- Zhu, M., Li, J., Chen, B., Mei, L., Yao, L., Tian, J., ... Li, H. (2015). The effect of calcium sodium phosphosilicate on dentin hypersensitivity: A systematic review and meta-analysis. *PLoS One*, 10(11), e0140176.