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In Vitro Effect of Innovative Desensitizing Agents on Dentin Tubule Occlusion and Erosive Wear

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Clinical Relevance

In-office desensitizing products are innovative and conservative methods for the decrease of dentin hypersensitivity due to its tubule occlusion properties. Depending on the diagnostic and pain related to the patient, the dentist is the professional responsible for indicating the appropriate treatment.

SUMMARY

Purpose: The purpose of this study was to evaluate the effects of four in-office desensitizing products on dentin tubule occlusion and erosive wear.

Methods: Dentin hypersensitivity was simulated by EDTA application for five minutes. The specimens were randomly allocated into five groups (n=11), according to treatment: No treatment - Control (C), Duraphat (DUR), Desensibilize Nano P (NP), ClinPro XT Varnish (XTV), and ClinPro White Varnish (CWV). They were then submitted to erosive/abrasive cycling for five days. After EDTA, treatment, and cycling, the specimens were analyzed with an environmental scanning electron microscope (ESEM) to verify the number of opened dentin tubules (ODT) which were counted by using ImageJ software, and with a profilometer to determine the surface curvature/loss. ESEM data were analyzed with two-way repeated measure analysis of variance and Tukey tests. For the profilometer, data were analyzed with Kruskal-Wallis, Tukey, and Mann-Whitney tests.

Results: After treatment, all groups showed lower ODT than the control, without signifi-

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cant differences between them. After cycling, the only group that showed lower ODT than the control was group XTV; however, it did not significantly differ from the other groups. For the profilometric analysis, there were significant differences in SL between the experimental times after treatment and after cycling for all groups ($p<0.05$). After cycling, no surface loss was detected in groups DUR and XTV, which presented a significantly different curvature than group NP and the control group, but not from group CWV. Surface loss was detected for the control and groups NP and CWV, without difference among them.

Conclusion: All desensitizing agents tested presented promising results concerning the obliteration of dentin tubules immediately after treatment. XTV was the only desensitizer capable of preventing the reopening of the tubules after the erosive/abrasive challenges. XTV and DUR presented a protective effect against dentin erosive wear.

INTRODUCTION

Dentin hypersensitivity (DH) is a common condition among patients around the world, and its characteristics are well known and widely reported in the literature.[1-3] There are many etiologic factors related to DH and denudation of the root surface with loss of the overlying cementum and periodontal tissues and the removal of the enamel because of wear being commonly associated with DH.[4] In this latter condition, it is likely that the impact of erosive acids and toothbrushing, depending on the degree of the abrasivity of the toothpaste, can open the dentin tubules, thus leading to DH.[5]

As the theory proposed by Brännström is the most widely accepted to explain the mechanism of pain in DH,[6-8] one of the main strategies to treat this condition consists in sealing the dentinal tubules, thus preventing fluid flow. There are currently a large number of commercially available desensitizing agents.[9-13] Among the in-office options that can be cited are adhesives, glass ionomer cements and sealants, as well as topical application of products containing sodium fluoride, tin fluoride, potassium nitrate, oxalates, calcium phosphate, oxalic acid (phytocomplexes), arginine/calcium carbonate, and bioglass (calcium phosphosilicate and sodium).[13]

Varnishes containing sodium fluoride are one of the most widely used products for the treatment of DH. Fluoride possibly acts by precipitating insoluble calcium fluoride within the dentinal tubules.[14] Clinical studies show that varnishes have beneficial effects in the treatment of DH[15,16]; however, according to a recent systematic review, its clinical effectiveness was considered only as limited.[2] Clinpro White Varnish (3M ESPE, St. Paul, MN, USA) is an example of a fluoride varnish, which, in addition to sodium fluoride (5%), contains tricalcium phosphate (TCP).[17] According to the manufacturer, the material releases calcium and fluoride ions when in contact with saliva, optimizing the formation of calcium fluoride up to a period of 24 hours. There is not much data evaluating the effectiveness of this varnish on tubule occlusion, especially under erosive and abrasive conditions. Tosun and others observed that at the end of a pH cycling the material remained at the dentin surface, partially occluding the dentinal tubules.[18]

Also containing calcium and phosphate in its composition, nano hydroxyapatite pastes (such as Desensibilize Nano P, FGM Dental Products, Joinville, SC, Brazil) were recently suggested as another treatment option for DH.[19-21] It was hypothesized that these pastes would promote tubule occlusion by the deposition and/or penetration of nano-sized particles onto or into the dentin tubules.[21] Clinical trials have shown that these pastes are effective against DH.[21-23] It has been observed that the particulate nano calcium phosphate exhibits mechanical obliteration properties twice as good as the traditional calcium phosphate compositions[24]; however, a recent *in vitro* investigation observed that the deposits formed by a hydroxyapatite paste is not resistant to acidic and mechanical challenges when compared to two varnishes.[25]

Another innovative product used for DH is a resin-modified glass ionomer, Clinpro XT Varnish (3M ESPE, St. Paul), which the manufacturer claims provides a specific coating of fluoride release for more than six months. The varnish also releases calcium and phosphate in a controlled manner.[26] In a clinical trial, it was observed that this material could reduce DH through a period of four weeks, being more effective than the resin-based material Gluma Desensitizer (Kulzer, Hanau, Germany).[25] An *in vitro* study found that it could sustain tubule occlusion even after seven days of erosive and abrasive challenges.[25] The manufacturer also claims that the product can be used to prevent dental erosion, but there is not much information about this in the literature.

Considering that these innovative desensitizing agents contain fluoride, calcium, and phosphate in their composition and that they are applied to the dentinal surface promoting a mechanical barrier, it is reasonable to suppose that they can also offer some protection against dentin erosive wear. This would be of utmost importance, because dental erosion and abrasion are strongly associated with the etiology and development of DH.[27] Thus, the objectives of this study were as follows: 1) to investigate, *in vitro*, the efficacy of four desensitizing agents in promoting tubule occlusion, as well as its resistance to erosive/abrasive challenges and 2) to evaluate their effect against dentin erosive wear.

The null hypotheses tested were the following: 1) there would be no difference among the products regarding the occlusion of dentinal tubules post treatment; 2) the products would not differ in their ability to promote tubule occlusion after an erosive/abrasive challenge; and 3) there would be no difference in dentin erosive loss among the groups after cycling.

METHODS AND MATERIALS

This study followed a completely randomized design with two experimental factors: desensitizing treatment was five levels: no treatment-negative control (C), Duraphat (DUR), Desensibilize Nano P (NP), ClinPro XT Varnish (XTV), and ClinPro White Varnish (CWV). Experimental time was three levels for tubule counting analysis (after EDTA, after treatment, and after cycling) and two levels for the profilometric evaluation (after treatment and after cycling). The factors were tested in an erosion-abrasion-remineralization model using human specimens (n=11 per subgroup). The response variables were dentin surface loss (SL, in micrometers, determined with optical profilometry) and the number of opened dentinal tubules (ODT; tubule counting was performed with ImageJ software [National Institutes of Health, Bethesda, MD, USA] on scanning electron microscopy images).

Sample Preparation

Eighty sound human third molars were collected. Human third molars were used in this study after the approval of the Local Ethics Committee (CAAE64008417.0.0000.0075). Teeth were cleaned with Gracey curettes 11-12 and 13-14 and Robinson's brush at low speed using a mixture of pumice and water, ending with an air/water spray. The roots were separated from the crowns using a water-cooled diamond disc (KG Sorensen, Barueri, São Paulo, Brazil) and stored in 0.1% thymol solution at 4°C until the beginning of the experiment. Dentin specimens 4 × 4 × 2 mm were cut from the roots and polished with a series of abrasive discs cooled with water (grit# 800, 1200, 2400, and 4000; Buhler, Uzwil, Switzerland). Between each polishing step, the samples were cleaned with distilled water in an ultrasonic cleaner (Digital Ultrasonic Cleaner CD-4820, Kondortech, São Carlos, Brazil) for five minutes to remove any debris. To simulate a hypersensitive dentin, the specimens were immersed in 17.5% EDTA solution for five minutes to remove the smear layer and open the dentin tubules. After that, the specimens were sonicated in distilled water (Digital Ultrasonic Cleaner CD-4820, Kondortech, Sao Carlos, Brazil) for five minutes to remove any debris.[28]

Treatments

After opening the dentinal tubules, the samples were randomly allocated into five experimental groups (n=11), according to their respective treatments (Table 1). All specimens received adhesive unplasticized polyvinyl chloride tapes (UPVC, Graphic Tape, Chartpak, Leeds, MA, USA) on their polished surfaces, leaving a central window of 4 × 1 mm exposed to receive the treatments and two lateral areas of 4 × 1.5 mm as control surfaces. The treatments were applied following the manufacturer's instructions, as described in Table 1. The negative control group received no treatment.

Abrasive/Erosive Challenges

For all groups, a modified five-day erosion-abrasion-remineralization model proposed by Scaramucci and others was used.[29] Erosive challenges were performed with a 0.3% citric acid solution (pH ≅ 2.6). The specimens were immersed in the citric acid solution for two minutes, four times a day, without stirring and at room temperature. Between the challenges, there was a 60-minute immersion in artificial saliva (1.649 mmol/L $CaCl_2 \cdot H_2O$, 5.715 mmol/L $KH_2 PO_4$, 8.627 mmol/L KCl, 2.950 mmol/L NaCl g/l.92 mmol/L Tris buffer, pH adjusted to 7 with HCl). After each episode of erosion, the specimens were rinsed with distilled water and gently dried with absorbent paper.

Tooth brushing was performed twice a day for 15 seconds in the middle of the first and last remineralization periods using electric brushes (Oral B Professional Care 3000f, Procter & Gamble, Cincin-

Table 1: *Description of the Densensitizing Products, Composition of the Agents Used in This Study, and Manufacturer's Application Instructions*

Product	Manufacturer	Composition	Protocol
Negative control			No surface treatment after sample preparation with EDTA
Clinpro white varnish	3M ESPE	Sodium fluoride (5%), tricalcium phosphate (TCP), xylitol	Apply in a thin layer over treatment area(s) with sweeping, horizontal brush strokes
Clinpro XT varnish	3M ESPE	Part A: glass particles of silanized fluoroaluminosilicate, HEMA, water, BIS-GMA, and silanized silica Part B: copolymer of polyalkenoic acid, water, HEMA and calcium glycerophosphate	Mix the components for 15 seconds; application of the varnish in a thin layer on tooth surface light-curing for 20 seconds and surface cleaning with a moistened pellet
Desensibilize nano P	FGM	Nanometric calcium phosphate, sodium fluoride (approximately 2%) and potassium nitrate 5%.	Active application for 10 seconds with felt disc at low speed; five minutes waiting time and excess removal
Duraphat	Colgate–Palmolive	Sodium fluoride (5% w/v) in an alcoholic solution of natural resins	With the surface dry, apply the material in a thin layer (brush, applicator or probe)

nati, OH, USA) equipped with a pressure alert that signals when the pressure reaches the value of 2.5 N. The brush head was positioned on a stabilizing device parallel to the surface of the specimens until the pressure alert was switched on. For the tooth for all groups, brushing was performed with a slurry made from Colgate Maximum Anti-caries Protection dentifrice and artificial saliva (1:3 w/w). The total exposure time of the specimens to the dentifrice slurries in each brushing episode was two minutes. A single operator performed the toothbrushing procedures. During the night, specimens were stored in a humid environment at 4°C.

Profilometric Analysis

After EDTA treatment, all specimens were analyzed with optical profilometry (Proscan 2100, Scantron Ltd, Venture Way, Taunton, UK) to discard specimens with curvature values higher than 0.3 μm, for sample standardization. The other readings were performed after treatment and at the end of cycling. The specimens were left to dry for 10 minutes before each profilometric analysis.[30] The instrument sensor scanned an area 2 mm long (x-axis) by 1 mm wide (y-axis), located at the center of the specimen. The equipment was set to go through 200 steps on the x-axis, with each step measuring 0.01 mm. On the y-axis, there were 20 steps measuring 0.05 mm each. Using a specific software (Proscan Application software version 2.0.17, Scantron Ltd), the lesion curvature or depth was calculated based on subtracting the average height of the test areas from the average height of the reference surfaces. The result was expressed in micrometers.

Environmental Scanning Electron Microscopy (ESEM) Evaluation

After EDTA treatment, all specimens were analyzed by ESEM (Hitachi TM3000, Hitachi, Tokyo, Japan) to verify, qualitatively and quantitatively, the number of opened dentin tubules. Representative micrographs were taken at ×2000 in the center of each specimen. No sample preparation was required. All specimens were qualitatively and quantitatively re-evaluated after treatments and cycling. In the qualitative assessment, micrographs had their surface characteristics evaluated and checked for patency and occlusion of the dentin tubules. The quantitative assessments were performed using the image analysis software program, ImageJ (National Institutes of Health), to standardize the counting of opened dentin tubules.[31,32]

Statistical Analysis

Data were analyzed for normal distribution and homoscedasticity with Shapiro-Wilks and Brown-Forsythe tests, respectively. For the ODT data, these assumptions were satisfied; therefore, comparisons among groups were performed with two-way repeated-measures analysis of variance (ANOVA) and Tukey tests. For surface loss, data did not follow a normal distribution; thus, comparisons among groups within experimental times were performed with Kruskal-Wallis and Tukey tests. Comparisons between experimental times (after treatment and

Table 2: *SEM Evaluation: Mean Values of Opened Dentinal Tubules*

Groups	After EDTA, Means (SD)	After Treatment, Means (SD)	After Cycling, Means (SD)
Control	129.09 (47.97) Aa	104.64 (29.42) Aab	76.00 (24.43) Ab
DUR	134.73 (53.12) Aa	0 (0) Bc	68.73 (50.73) ABb
XTV	129.64 (48.02) Aa	0 (0) Bc	31.91 (44.43) Bc
CWV	132.18 (46.48) Aa	0 (0) Bc	74.09 (42.12) ABb
NP	136.54 (49.72) Aa	4.73 (9.06) Bc	74.73 (31.85) ABb

In columns, uppercase letters indicate differences among groups at each experimental time. In rows, lowercase letters indicate differences amongst experimental times for the same treatment group.

after cycling) within groups were performed with the Mann-Whitney test. The software Sigma plot 12 (Systat Software Inc, San Jose, CA, USA) was used for all calculations. The significance level was set at 5%.

RESULTS

For ODT, there were significant differences among the levels of the factors treatment ($p<0.001$) and experimental times ($p<0.001$) and in the interaction between them ($p<0.001$).

Relative to the factor treatment after EDTA, there were no significant differences in the number of ODT among the groups. After treatment, all the groups showed lower ODT than the control, without significant differences between them. After cycling, the only group that showed lower ODT than the control group was group XTV; however, it did not significantly differ from the other groups. All the groups, except XTV, did not significantly differ from the control.

Regarding the factor experimental times, for the groups CWV, DUR, and NP, ODT was lower after treatment, followed by after cycling and then after EDTA. For the control, the lowest ODT was observed after cycling, but it was no different from ODT after treatment, which in turn did not differ from after EDTA. For group XTV, the highest ODT was observed after EDTA, and the ODT from after treatment and after cycling was not significantly different.

The means (SD) of open dentin tubules according to each DH treatment in all experimental times are shown in Table 2. Representative images of the groups in each time, are shown in Figures 1 and 2. Also, an example of ImageJ software (National Institutes of Health) analysis of opened dentinal tubules may be seen in Figure 2.

For the profilometric analysis, there were significant differences between the experimental times after treatment and after cycling for all groups ($p<0.05$).

After treatment, groups DUR, XTV, and CWV presented the highest curvature, with no significant difference among them, indicating the presence of a layer of the material on their surface. The curvature of the control group did not differ from that of group NP, which in turn was no different from group CWV. After cycling, no surface loss was detected in groups DUR and XTV, which presented significantly different curvature than group NP and the control group, but not from group CWV. Surface loss was detected for the control group and groups NP and CWV, with no differences among them.

Medians (interquartile interval) of dentin surface loss for the groups in each experimental time are shown in Table 3.

DISCUSSION

In the present study, all desensitizer agents were capable of obliterating the dentinal tubules immediately after their application, making them suitable approaches for the treatment of DH. In view of this result, the first null hypothesis was accepted. However, only group XTV showed resistance to the erosive/abrasive challenges, sustaining tubule occlusion after cycling; therefore, the second null hypothesis was rejected.

Table 3: *Profilometer Evaluation: Median Values of Dentin Surface Loss*

Groups	After Treatment, Median (25%/75%)	After Cycling, Median (25%/75%)
Control	0.13 (0.07/0.25) C	−7.653 (−9.18/−5.72) B
DUR	151.89 (108.56/311.89) A	3.769 (−2.00/56.97) A
XTV	118.67 (75.19/153.12) A	0.143 (−4.44/110.72) A
CWV	66.21 (48.35/132.51) AB	−4.110 (−5.51/−2.79) AB
NP	2.84 (1.20/6.68) BC	−5.374 (−6.34/−4.46) B

All test groups showed statistically significant differences between experimental times after treatment and after cycling ($p<0.05$).

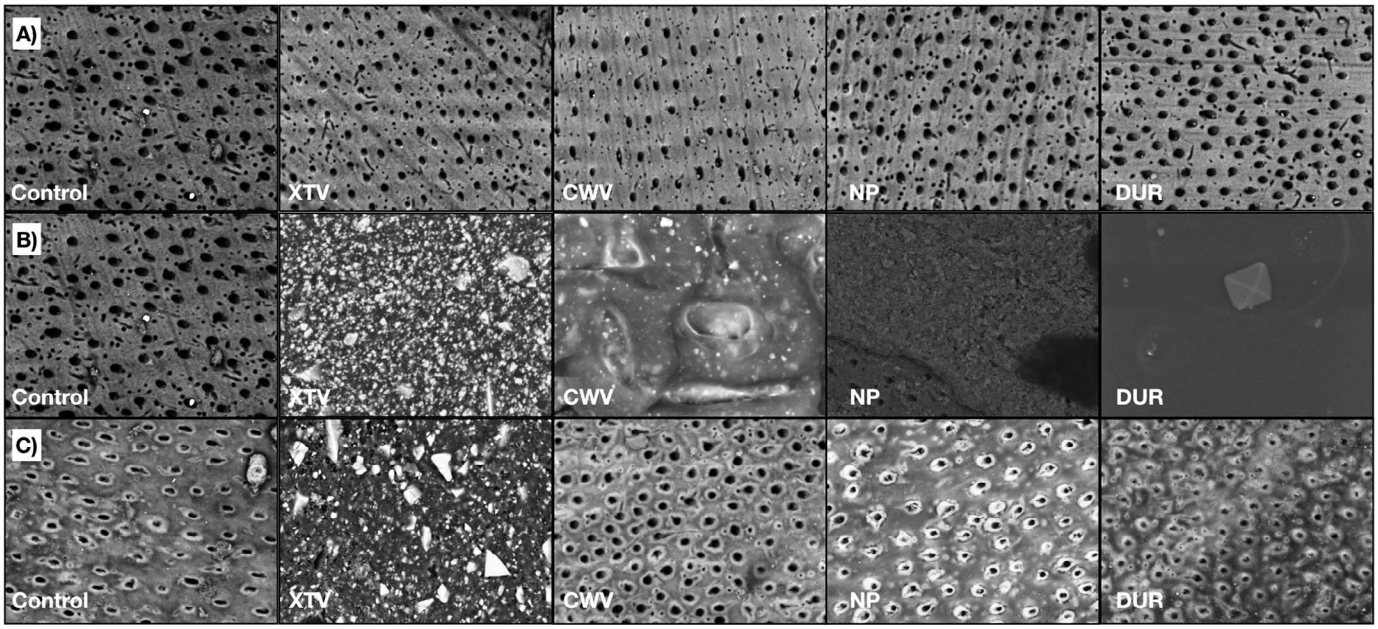

Figure 1. *(A) Dentin surface after EDTA. Control group, XTV group, CWV group, NP group, and DUR group. (B) Dentin surface after treatment. Control group, XTV group, CWV group, NP group, and DUR group. (C) Dentin surface after erosion/abrasion cycling showing a visibly worn treatment layer with dentin exposure. Control group, XTV group, CWV group, NP group, and DUR group.*

ClinPro XTV varnish (XTV) is a resin-modified glass ionomer and, as such, can be used as a sealant for class V lesions. Its efficiency may be explained by the action of its components, such as the polyalkenoic acid copolymer, which provides chemical adhesion to dentin by ionic bonding with the hydroxyapatite calcium, the prevalent mineral of dentin.[33] The lack of surface loss observed for XTV after cycling could be due to the presence of a coating of material over the dentinal surface, which prevented direct acid contact, and the action of toothbrushing. Compared with the profilometric analysis performed after treatment, the surface curvature of this group was reduced after cycling, suggesting that, under the conditions of this study, the material underwent some wear, but not up to a point of allowing surface loss. Nevertheless, it should be mentioned that, in a few specimens, a complete detachment of the

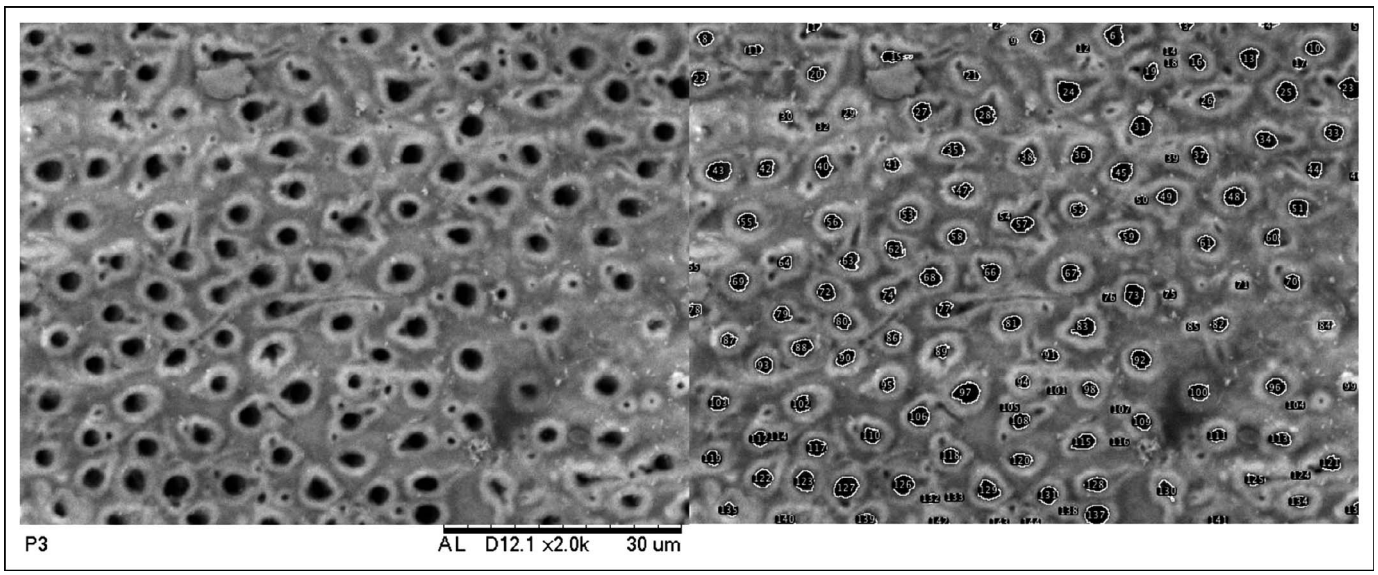

Figure 2. *Example of ImageJ software count of opened tubules.*

material off the surface was observed after cycling, thus producing the large variation found in the data from this group. In view of these results, further investigations are needed to evaluate the impact of acidic and mechanical challenges on the adhesion of the material to the dentin, especially in the long term. A previous investigation observed that the shear bond strength of XTV to dentin was lower than some resin-based coatings.[34]

In addition to promoting a physical barrier at the dentin surface, XTV is able to release fluoride, calcium, and phosphate to the aqueous medium; the two latter are a result of the presence of calcium glycerophosphate in its composition.[35,36] Such mechanisms could be observed in previous studies.[26,37] In the investigation of Virupaxi and others,[37] fluoride released in artificial saliva by XTV was evaluated for a period of six months. The authors observed that the material released 18.78 ppm of F within the first week, a value that dropped to almost half after one month, but that was kept relatively stable for six months. It must be noted, however, that in this study, the material was applied to the enamel and not the dentin. In Zhou and others,[26] the authors observed that XTV was more effective than a fluoridated varnish in promoting enamel remineralization, an observation that was attributed to the presence of calcium and phosphate in its formulation. In view of these findings, in the case of the present study, the authors also suggest that XTV released calcium, phosphate, and fluoride to the acidic solution during the erosive challenge, increasing its saturation regarding tooth minerals, thereby reducing the demineralization rate. Similarly, it could have released these ions when in the saliva, enhancing remineralization.

Another material that was able to significantly reduce dentin loss after cycling, although not being able to promote a significant tubule occlusion, was the fluoridated varnish Duraphat (DUR). A NaF varnish was included in the present study as a positive control, as it is commonly applied in dental practices for the treatment of DH.[2] Its fluoride-releasing mechanism is widely described in the literature as the creation of a barrier by precipitating calcium fluoride (CaF_2) on the tooth surface, which blocks the patent dentinal tubules and hence reduces permeability and hypersensitivity.[15,16,38,39] Similar to XTV, the ability of DUR to reduce dentin loss can be attributed to its persistence at the dentin surface until the end of the cycling, although some detachment was also observed. In addition, the deposition of a CaF_2-like material may also have contributed to the dentin's protection.[40] However, the occluding ability of DUR was not significantly higher after cycling than the control. It could be suggested that not only complete, but also partial, detachment of this varnish occurred throughout the cycling, which resulted in the opening of some dentinal tubules. To corroborate this finding, the number of ODT for this group was lower after cycling than after EDTA, indicating that at least a portion of the dentinal tubules remained occluded by the varnish. This theory is in agreement with the observations of West and others, who stated that the effectiveness of varnishes in DH appears to be limited to the duration in which they remained on the tooth surface,[2] which was estimated to be 24 hours *in situ*. However, it must be mentioned that, in DUR, although the software considered some tubules opened, they seemed to have their diameter reduced compared with the control (Figure 3). This must be the result of the deposition of the CaF_2-like material, which may exert a clinical impact on DH.

Due to the presence of calcium and fluoride in its formula, it was expected that CWV would behave better than DUR regarding tubule occlusion, possibly by the formation of more CaF_2-like deposits, but this was not observed. Although CWV was able to promote some tubule occlusion that did not return to the values after EDTA after cycling, at that time its number of ODT did not differ from the control.[17,41,42] Similar to DUR, it can be hypothesized that the cycling promoted partial removal of CWV from the surface, exposing dentin tubules. Nonetheless, opposite of DUR, the tubules with CWV do not appear with a reduced diameter. Regarding surface loss, it can be suggested that CWV has lower adhesion to the dentin compared with DUR due its lack of protection against erosive wear. In this context, it must also be taken into account that the layer of material induced by CWV was thinner than DUR, as can be seen in the surface profile obtained after treatment. This can also influence the comparison. Concerning the formation of CaF_2-like deposits, in previous investigations, the amounts of fluoride, calcium, and phosphorus released by different varnishes in lactic acid were evaluated. It was observed that within 24 hours of exposure, the calcium release of CWV was no different from DUR, but after 48 hours, it was significantly higher than DUR. Phosphorus release was very low for CWV, but its fluoride release was almost four times higher than DUR in both experimental times.[43] The authors suggested that the low phosphorus found for CWV could be related

to the low amount of beta tricalcium phosphate (TCP) added to the varnish or to the low solubility of tricalcium phosphate. If that is indeed the case, it could also help to explain why CWV did not behave better than DUR under the conditions of this investigation.

In this study, Desensibilize Nano P (NP) was effective in occluding the dentinal tubules immediately after application; however, it did not show a resistance to the cycling procedures, despite the manufacturer's claim that NP imparts greater stability and resistance to the acid challenge because of the crystalline form of its mineral content. NP resulted in a heterogeneous pattern of obliteration, also observed by Canali and others.[36] This might have occurred because of its lower viscosity, thus resulting in a thinner layer of product over the tooth surface. Another explanation would be related to the lower concentration of fluoride in NP compared with the other products tested. It has less than half of the fluoride content than Duraphat and ClinPro White Varnish (9000 versus 22,600 ppm F), which may have impacted its ability to form CaF_2-like material, as higher concentrations induce more deposition of these globular structures.[44] It is possible that a greater number of applications would result in a more effective occlusion, as shown in a previous clinical study.[45] This result is in accordance with a previous *in vitro* report, which showed that NP was less effective in reducing dentin permeability than XTV and a fluoridated varnish immediately after application and after seven days of erosive-abrasive cycling.[43] Nevertheless, that study is in disagreement with a previous investigation that tested a different nano hydroxyapatite paste and found that the precipitates formed by the paste were resistant to an erosive challenge of one minute with 6% citric acid.[46] The more aggressive erosive challenge performed in the present study (two-minute immersion in 0.3% citric acid, four times a day for five days) might explain these different results. Clinically, NP demonstrated the ability to reduce DH for up to three months.[21] In this case, the role of fluoride and potassium nitrate as active agents included in the NP should also be considered. It has been discussed that potassium salts are able to inactivate intradental nerves, contributing to the reduction of DH. However, this principle has never been confirmed.[21] Concerning surface loss, NP did not show any protective effect. Not much data exists about the effect of nano hydroxyapatite pastes on dental erosion.

This study used an erosion-abrasion cycling model with the main objective of simulating the clinical situation of individuals reporting DH, because dietary acids and toothbrushing are known to be capable of opening and enlarging dentin tubules, initiating the condition, or reducing the effectiveness of the treatments. The cycling protocol was adapted from Scaramucci and others and attempted to simulate the situation of patients with a high frequency of acidic beverage consumption.[29,47] The literature has reported that in the mouth, the maximum time that the pH remains low is about two minutes, and extrapolation of this condition may modify the eroded surface to an unrealistic state.[47,48] Toothbrushing abrasion occurred twice daily, in an endeavor to simulate a realistic daily oral hygiene habit.[27] Toothbrushing was performed with an electric toothbrush, fixed in a specific device that standardized the brush movement over the specimen and controlled the brushing force at 2.5 N, which is within the range of force recommended for erosion-abrasion studies.[31,49] Colgate Maximum Anti-caries Protection was chosen for toothbrushing because it is a regular 15,000 ppm F (as NaF) toothpaste, without any desensitizing claim. The slurry used was prepared with artificial saliva, which has calcium in its composition. This could enhance the action of the desensitizing agents tested, as well as explain the partial occlusion of some tubules in the control group after cycling compared with after EDTA. Because artificial saliva was used, the enzymatic and microbiological effects of human saliva could not be expected.

To properly simulate clinical conditions, the desensitizer agents were applied according to the manufacturers' instructions. A limitation of this methodology was that, as each product presented a different consistency, the layers applied on each specimen were not uniform and standardized. In this sense, care should be taken when extrapolating the findings of this study to the clinical scenario, but it can provide information and a basis for further analysis under more realistic clinical conditions.

CONCLUSION

Considering the limitations of this *in vitro* investigation, it can be concluded that all desensitizing agents tested presented promising results concerning the obliteration of dentin tubules immediately after treatment. ClinPro XT Varnish was the only desensitizer capable of inhibiting the reopening of the tubules after erosive/abrasive challenges. Clin-

Pro XT Varnish and Duraphat presented a protective effect against dentin erosive wear.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the approval of the Local Ethics Committee guidelines and policies of the Faculty of Dentistry of the University of Sao Paulo. The approval code for this study is CAAE64008417.0.0000.0075.

Conflict of Interest

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

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