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Effect of in-office desensitizers containing calcium and phosphate on dentin permeability and tubule occlusion

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ABSTRACT

Objectives: To evaluate the performance of calcium/phosphate desensitizing agents when used for dentin permeability and tubule occlusion.

Methods: 1 mm-thick dentin specimens were immersed in 17% EDTA solution and allocated into 7 groups: 1. Clinpro White Varnish, 2. Clinpro XT Varnish, 3. Teethmate Desensitizer, 4. Desensibilize Nano P, 5. Nupro prophylaxis paste, 6. Duraphat (reference product), and 7. Control (no treatment). After treatment, specimens were submitted to erosion-abrasion cycling for 5 days. Dentin permeability was assessed by hydraulic conductance (n = 10) and environmental scanning electron microscopy ESEM (n = 8) post-EDTA, post- treatment and post-cycling. The percentage of permeability (%Lp) was calculated post-treatment and post-cycling. ImageJ software was used to obtain the number of open dentin tubules (ODT) in the micrographs. Data were statistically analyzed ($\alpha = 0.05$).

Results: Post-treatment, the %Lp values of all treatments were significantly lower than the control, with Nupro presenting higher %Lp values than Duraphat. All the groups presented significantly lower %Lp values in the post-treatment in comparison with the post-cycling period, except the control. Post-treatment, all groups showed lower numbers of ODTs than the control, except Nupro. Clinpro WV, Clinpro XT, Duraphat and Nano P presented the best results. Post-cycling, there was no significant difference among groups. Clinpro WV and Duraphat presented lower numbers of ODTs post-treatment than they had post-cycling, and the control had a higher number of ODTs post-treatment than they had post-cycling.

Conclusions: Most treatments were efficient in reducing both dentin permeability and number of ODTs after treatment; however, none of the products were able to resist the erosive-abrasive challenges.

1. Introduction

Dentin hypersensitivity (DH) is a common oral finding, with reports of prevalence varying widely from 1.34% to 92.1% [1,2], depending on the population and the methodology used [1,3]. DH has been characterized as a stimulus induced pain that occurs when the dentin becomes exposed to the oral environment as a result of open and patent dentin tubules. In this case, the incidence of thermal, tactile, evaporative and osmotic changes at the dentin surface can cause movement of the fluid inside its tubules, stimulating the nerve fibers near the dentin/pulp division, thus triggering a painful sensation [4,5]. While gingival recession caused by different processes is the most common etiological factor of root dentin exposure, several chemical and mechanical factors can contribute to the loss of tooth substance above the cement-enamel junction, influencing the occurrence of DH [3]. Dental erosion is known to play an important role in the onset of DH, because erosive acids can open and enlarge the dentinal tubules [6,7]; however, a synergistic action with toothbrushing is most likely to occur, with both processes acting together to promote surface loss and opening of the dentinal tubules [3].

A classical scanning electron microscopy (SEM) study showed that hypersensitive dentin has approximately eight times the number of open dentinal tubules that have twice the diameter when compared with those in non-sensitive dentin [8]. Considering this finding and the pain mechanism proposed for DH, it can be assumed that any treatment capable of reducing the hydraulic conductance of dentin by a

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mechanism of tubular occlusion may also be capable of reducing the pain caused by DH. Nowadays, there are several desensitizing agents that act through this mechanism of action, and their effectiveness is thought to be related to their ability to withstand the chemical and mechanical challenges present at the oral environment [3,9]. These agents can be added to products for at home or in-office use.

In-office desensitizing products can be found in the form of sealants, varnishes, gels, prophylactic pastes or adhesive systems [10]. These products can contain a variety of active ingredients, such as fluoride, oxalates, potassium nitrate and calcium phosphates [3,4]. Calcium phosphate compounds are interesting/appealing systems, due to their biocompatibility [11] and remineralization capacity [12]. At present, there are several calcium phosphate containing products available on the market, but there are few data comparing their effects on the hydraulic conductance of dentin. The pulp pressure simulation method increases the reliability of treatment evaluations, because microscopic analyses of tubule occlusion does not always indicate reduction in permeability, as the deposits created by the treatments may not be firmly bound [13].

In view of the abovementioned, the aim of this study was to evaluate dentin permeability and tubule occlusion after the application of several in-office desensitizing treatments containing calcium and phosphate, and to analyze their resistance to erosive and abrasive challenges. The null hypotheses were: 1) the groups would not differ regarding their ability to reduce dentin permeability or to promote tubule occlusion after application; 2) there would be no difference in dentin permeability and tubule occlusion among the groups after a 5-days erosive-abrasive challenge.

2. Materials and methods

This study was based on a completely randomized design with two experimental factors: 1. Desensitizing treatment, at seven levels (Table 1): Clinpro White Varnish; Clinpro XT Varnish; Teethmate Desensitizer; Desensibilize Nano P; Nupro prophylaxis paste; reference product without calcium and phosphate (Duraphat); and a control group (without treatment); 2. Experimental time intervals, at two levels in the hydraulic conductance analysis: post-treatment and post-cycling, and at three levels in environmental scanning electron microscopy (ESEM): post-EDTA, post-treatment and post-cycling. The treatments were tested in an erosion-abrasion model using human dentin specimens (n = 10 for hydraulic conductance and n = 8 for ESEM). The response variable of the hydraulic conductance analysis was reported as the permeability percent (%Lp, determined with respect the post-EDTA permeability), and that of the ESEM was the number of open dentin tubules (ODT), evaluated quantitatively by the ImageJ software, and qualitatively by visual analysis.

2.1. Specimen preparation

This study was conducted after approval by the local Research Ethics Committee of the institution (Process #2.340.539). Seventy sound human molars were used, and the crowns were separated from the roots. The crowns were used to prepare 1 mm-thick dentin discs for use in the hydraulic conductance analysis. Two sections 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 to remove the pulp horns and the occlusal enamel. From the roots, dentin slabs (4 mm × 4 mm × 1 mm) were prepared for evaluation by ESEM. Two perpendicular and two horizontal sections to the long axis of the tooth were made in the middle region of the roots, with a distance of approximately 4 mm between them. All sections were made using a precision cutting machine (Isomet 1000, Buehler Ltd, Lake Buff, Illinois, USA). The test surfaces of the specimens 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 and slabs reached a thickness of 1 mm. This procedure also removed any occlusal enamel that might have remained on the discs. The thickness of the discs and slabs 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. To simulate DH, the specimens were immersed in 17% EDTA solution (pH 7.4) for 5 min, for the purpose of opening the dentinal tubules.

2.2. Post-EDTA dentin permeability and ESEM analyses

After immersion in EDTA, the specimens were rinsed with distilled water and stored in a humid environment at 4 °C until their post-EDTA permeability was evaluated by hydraulic conductance, and the ESEM analysis. The specimens were then randomly allocated into the experimental groups, i.e., n = 10 for the permeability analysis, and n = 8 for the ESEM evaluation.

2.3. Application of the treatments

The specimens received the in-office treatments according to the protocols established in the literature or the manufacturer's recommendations, as described in Table 1. The specimens were then stored at 4 °C in a humid environment, and a new evaluation of the dentin permeability and ESEM analysis was performed (post-treatment).

2.4. Erosion-abrasion cycle

For the purpose of evaluating the resistance of the desensitizing

Table 1
In-office desensitizing treatments: description of the manufacturer, composition of the product and protocol of application.

Group/Treatment	Manufacturer	Composition	Protocol of application
Negative control	–	–	No surface treatment
Clinpro™White Varnish	3M ESPE	Sodium fluoride (5%), tricalcium phosphate (TCP), xylitol	Apply a thin layer over treatment area(s) with sweeping, horizontal brush strokes
Clinpro™XT Varnish	3M ESPE	Part A: glass particles of silanized fluoro-alumino-silicate, HEMA, water, BIS-GMA, and silanized silica Part B: copolymer of polyalkenoic acid, water, HEMA and calcium glycerophosphate	Mix the components for 15 s; application of the varnish in a thin layer on tooth surface, light-curing for 20 s and surface cleaning with a moistened pellet
Teethmate™ Desensitizer	Kuraray	Calcium tetra phosphate, anhydrous dicalcium phosphate and water	One drop of liquid plus one shallow scoop of powder (for each tooth): apply to the desired area for 30 s and wash
Desensibilize Nano P	FGM	Nanometric calcium phosphate, sodium fluoride (approximately 2%) and potassium nitrate 5%.	Active application for 10 s with felt disc at low speed; five min waiting time and excess removal
Nupro	Dentsply	Bioactive glass, NovaMin® (15% calcium sodium phosphosilicate), hydrated silica, glycerin, water, bicarbonate, sodium saccharine	Mix the paste with a prophy angle, running the angle at low speed. Evacuate saliva and apply the paste leaving in contact for 60 s. Rinse full mouth with water spray.
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)

Table 2
Description of the procedures involved in 5-days erosion-abrasion cycle.

	Procedures
1	Erosion (0.3% citric acid, pH = 2.6) for 2 min Remineralization (artificial saliva) for 30 min Toothbrushing (2 min exposure to the slurry; 15 s actual brushing) Remineralization (artificial saliva) for 30 min
2	Erosion (0.3% citric acid, pH = 2.6) for 2 min Remineralization (artificial saliva) for 60 min
3	Erosion (0.3% citric acid, pH = 2.6) for 2 min Remineralization (artificial saliva) for 60 min
4	Erosion (0.3% citric acid, pH = 2.6) for 2 min Remineralization (artificial saliva) for 30 min Toothbrushing (2 min exposure to the slurry; 15 s actual brushing) Remineralization (artificial saliva) overnight period

treatments to chemical and mechanical challenges, the specimens underwent a 5-day erosion-abrasion cycling protocol (Table 2). Each cycle consisted of 2 min immersion in citric acid (0.3%, natural pH of ˜2.6), followed by 60 min of immersion in artificial saliva (0.213 g/l $CaCl_2 \cdot 2H_2O$; 0.738 g/l KH_2PO_4; 1.114 g/l KCl; 0.381 g/l NaCl; 12 g/l Tris buffer, pH adjusted to 7.0 with KOH) [14], under constant agitation (35 rpm, orbital shaker, AI9000IB, BrILabs), 4 times a day. Thirty minutes after the first and the last acid challenges, the specimens were brushed in an automatic brushing machine for 15 s (45 cycles, each cycle was considered a back and forth brush movement, at a load of 2 N). Brushing with standard brushes (Tek, soft bristles, Johnson & Johnson, Brazil), was performed with a toothpaste and artificial saliva slurry, in a ratio of 1 part of toothpaste to 3 parts of artificial saliva (w/w).

A conventional fluoridated toothpaste (Colgate Maximum Caries Protection, Colgate Palmolive, Brazil, sodium monofluorophosphate, 1450 ppm F^-) was used. The total time of exposure to the toothpaste suspension was 2 min. The specimens were rinsed with distilled 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 artificial saliva, under constant agitation, until the beginning of the next cycle. The citric acid solution was replaced after each demineralization episode (4 times per day) and the artificial saliva was exchanged before the beginning of each cycle (once daily). After the 5 days of erosion-abrasion cycling, the final analyses of dentin permeability were performed by hydraulic conductance and ESEM (post-cycling).

2.5. Hydraulic conductance analysis

For dentin permeability analysis by hydraulic conductance, the dentin disc specimens were placed in the chamber of the machine (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, thereby simulating intrapulpal pressure [15]. The occlusal surface was marked to ensure that the readouts were made in the same area.

The system was kept 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 tubing (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 into flow volume (µL m^1), which was transformed into hydraulic conductance (Lp; $mim^{-1}\ cm^2 cmH_2O^{-1}$). Hydraulic conductance takes into account the area of the specimen through which the water passed (area = 0.58 mm^2), the pressure in the system and the fluid flow volume. The dentin permeability of each specimen (%Lp) was expressed as a percentage of the post-EDTA hydraulic conductance (considered 100% permeability).

2.6. Environmental Scanning Electron Microscopy (ESEM)

In the Post-EDTA, post-treatment, and post-cycling periods, all specimens (n = 8) were qualitatively and quantitative analyzed by ESEM (Hitachi TM3000, Hitachi, Tokyo, Japan). Representative micrographs were taken at 2,000x magnification in the center of each specimen. No sample preparation was required. For the quantitative analysis, the number of open dentin tubules (ODT) was counted using the ImageJ software, as previously described [16,17].

2.7. Statistical analyses

Normality and homoscedasticity of the %Lp and number of ODT data were checked with the Shapiro-Wilk and Brown-Forsythe tests, respectively. Since %Lp data did not follow a normal distribution, they were converted to Log, and then evaluated by two-way repeated measures ANOVA and Tukey tests, considering a significance level of 5%. The software SigmaPlot 13.0 (Systat Software) was used for all calculations. Qualitative description was given of the micrographs obtained by ESEM.

3. Results

3.1. Hydraulic conductance analysis

There were significant differences among the levels of the factor treatment ($p = 0.005$), between the levels of the factor experimental time ($p < 0.001$), and in the interaction between factors ($p = 0.001$). Means (standard deviations) of the groups at both experimental time intervals are shown in Fig. 1.

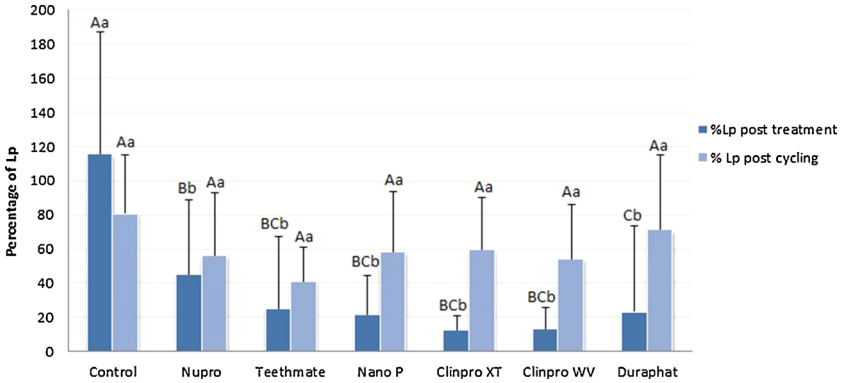

Fig. 1. Means and standard deviations of the groups at both experimental time intervals. Different Capital letters denote significant difference among groups, within each experimental time ($p < 0.05$). Different lowercase letters imply significant difference between times, within groups ($p < 0.05$).

Relative to the factor treatment, post-treatment, the %Lp values of all treatments were significantly lower than those of the control group ($p < 0.001$ for Duraphat, Clinpro WV, Clinpro XT, and Teethmate; $p = 0.002$ for Nano P; and $p = 0.38$ for Nupro). Duraphat showed no significant difference from groups Clinpro WV, Clinpro XT, Teethmate and Nano P ($p > 0.05$), but differed significantly from Nupro. Post-cycling, there were no significant difference among groups ($p > 0.05$).

As regards the factor experimental time, the only group that showed no difference in %Lp post-treatment in comparison with the post-cycling period was the control group ($p = 0.558$). All the other groups presented significantly higher %Lp values post- cycling than they did post-treatment (Clinpro XT: $p < 0.001$; Clinpro WV: $p < 0.001$; Teethmate: $p = 0.004$; Nano P: $p = 0.001$; Duraphat: $p < 0.001$; Nupro: $p = 0.037$).

3.2. ESEM evaluation

3.2.1. Quantitative assessment

There were significant differences among the levels of the factor treatment ($p < 0.001$), between levels of the factor experimental time ($p < 0.001$), and in the interaction between these factors ($p < 0.001$). Means (standard deviations) of the number of ODTs for the groups at both experimental time intervals are shown in Fig. 2.

Relative to the factor treatment, post-EDTA, the number of ODTs of all groups did not differ significantly. Post- treatment, the number of ODTs of all groups was lower than that of the control ($p < 0.05$), except for Nupro ($p = 0.121$). Clinpro W, Clinpro XT and Duraphat that showed the lowest ODT value, without differing significantly from Nano P ($p > 0.05$), which in turn did not differ from Teethmate ($p = 0.224$) and Nupro (0.099). Post- cycling, there were no significant differences among the groups ($p > 0.05$).

Regarding the factor experimental time, the control was the only group that showed no significant differences in ODT values when the post-EDTA values were compared with those of the post-treatment period ($p = 0.35$). The other groups presented significantly lower ODT values post- treatment in comparison with the post-EDTA period (Clinro XT: $p < 0.001$; Clinpro WV: $p < 0.001$; Teethmate: $p < 0.001$; Nupro: $p = 0.001$; Nano P: $p < 0.001$; Duraphat: $p < 0.001$). The control presented lower ODT values post-cycling than they did in the post-treatment period ($p = 0.017$). Only Clinpro WV ($p < 0.001$) and Duraphat ($p = 0.013$) showed lower ODT values post- treatment than they did in the post-cycling period. For the other groups, there were no significant differences between the post- treatment and post-cycling values.

3.2.2. Qualitative assessment

Representative micrographs of all groups post-EDTA, treatment, and cycling are presented in Fig. 3. As observed in the post-EDTA micrographs, the dentin tubules were open and exposed for all groups. Post-treatment, a large number of tubules were still visible for the control. On the other hand, for Clinpro XT and Clinpro White Varnish, the dentin tubules were not visible, and there seemed to be a layer of material covering the surface. For groups Duraphat, Desensibilize Nano P, Nupro prophylaxis paste and Teethmate, it was possible to observe areas covered by the material, but there were also areas of exposed dentin tubules. Post-cycling, the number of visible tubules increased for all groups, but remnants of materials were still visible for some, especially for Clinpro XT. For the control, it was possible to observe open dentin tubules with some deposits on the surface.

4. Discussion

In this study, two methods of analyses were used to verify the potential of the products to promote tubule occlusion. One was hydraulic conductance, which was used to evaluate dentin permeability by measuring the fluid flow movement across the specimen, and the other, ESEM evaluation of the specimen surfaces followed by counting the number of open dentin tubules (ODT). Although both methods presented limitations, they seemed to be complementary when evaluating the in vitro the effectiveness of desensitizing agents. The dentin permeability test had the advantages of simulating a constant intra-pulpal pressure, and enabling the readouts to be taken at the same place, promoting a better reproducibility of the measurements [18], and allowing each specimen to serve as its own control. The ESEM analysis followed by tubule counting was also an interesting assessment, because it was able to show changes in the dentin surface morphology caused by the treatments in the different experimental time intervals, without requiring any sample preparation. To allow a more accurate comparison, the images were taken in same place of the specimen. The use of a software program to perform tubule counting was also an interesting approach, because it reduced the subjectivity of the analysis [16,17,19]. The drawback of these two analysis was the small area evaluated (0.58 mm^2 for the permeability analysis and 0.0048 mm^2 for the ESEM evaluation); however, we endeavored to overcome this limitation by applying the treatments as uniformly as possible across the entire dentin surface and to conduct the analyses in the same place of the specimen. Another point was that the permeability analysis did not make it possible to verify whether a reduction in the diameter of dentin tubule occurred, and this could also have been implicated in the relief of DH [8].

According to our results, all treatments were able to reduce dentin

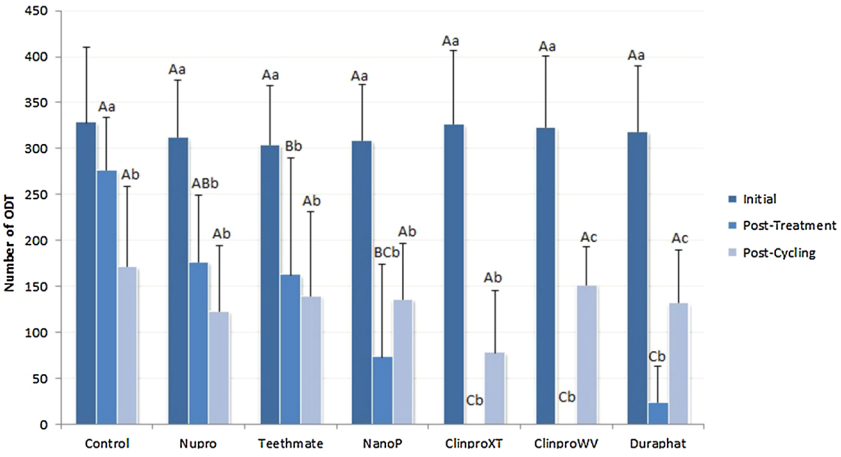

Fig. 2. Means and standard deviations of the groups at both experimental time intervals. Different Capital letters denote significant difference among groups, within each experimental time ($p < 0.05$). Different lowercase letters imply significant difference between times, within groups ($p < 0.05$).

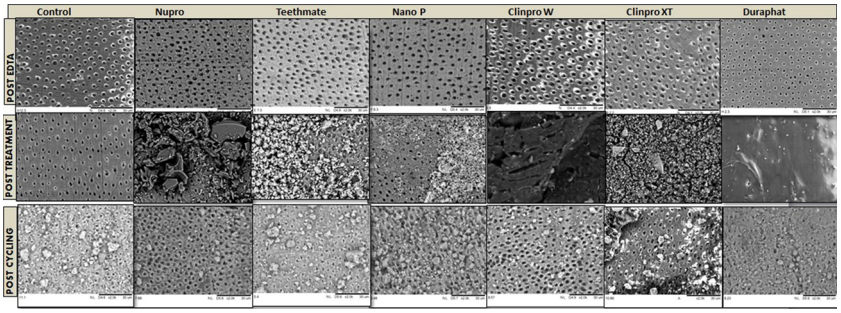

Fig. 3. Representative micrographs of all groups post- EDTA, treatment, and cycling, at 2,000x magnification.

permeability and the number of ODTs after application when compared with the control, except for Nupro, in which the number of ODTs did not differ significantly from the those of the control. In view of this, our first study hypothesis was rejected.

Duraphat, Clinpro XT, Clinpro WV, Teethmate and Nano P were the groups with the lowest dentin permeability after treatment. In these groups, the treatments were able to reduce dentin permeability in a range of 75–87%. The fact that the pastes (Teethmate and Nano P) showed similar reduction in permeability in comparison with the varnishes (Duraphat, Clinpro XT, Clinpro WV) was somewhat surprising, considering that the varnishes also behaved as coatings, which in theory would result in a more efficient sealing of the dentinal tubules. Nevertheless, the small area (0.58 cm^2) evaluated in the permeability analysis could be the factor responsible for this result, because it did not allow proper differentiation between the treatments that more homogenously covered the surface, from those that only left dispersed/scattered deposits on it [15]. This hypothesis could be corroborated by the ESEM micrographs, in which the varnishes seemed to have created a layer over the surface of the specimens. Quantitatively and qualitatively, for Clinpro XT, Clinpro WV and Duraphat varnishes, almost no dentin tubules could be counted, or were visible enough to be counted. On the other hand, Teethmate presented a higher number of ODTs than the varnishes. Nupro prophylaxis paste also presented distinct results in both analyses. In the permeability test, it showed lower permeability than the control, but in the ESEM analysis, it did not differ significantly from the control. The reason for these discrepant results could be the same as that stated for Teethmate, considering the more heterogeneous distribution of the deposits of this product seen on the dentin surface.

Post- cycling, there were no significant differences in dentin permeability and the number of ODTs among groups, implying that the treatments were not able to resist the 5 days of erosive and abrasive challenges; thus, our second null hypothesis was accepted.

At this experimental time interval, a superior effect of the varnishes was also expected, because of their greater adhesion to the dentin surface, in addition to their release of fluoride, calcium and phosphate. The varnishes tested in this study were Clinpro WV, Clinpro XT and Duraphat, the latter being a fluoride varnish chosen as a reference product (without calcium and phosphate), as it is commonly recommended for the DH treatment [9]. Duraphat has a high concentration of sodium fluoride (5%). When in contact with the dentinal surface, it results in the formation of a layer of CaF_2, which is capable of occluding the dentinal tubules [10,20]. This occlusion would also result from the presence of the varnish itself on the dentin surface, blocking the tubules for as long as it remained attached to the tooth [3].

Clinpro WV is a fluoride varnish (5%) containing tri-calcium phosphate. Due to the presence of calcium in its formula, a better performance of this material would have been expected in comparison with Duraphat in the post-cycling period, because of its potential for higher precipitation of CaF_2-like material [10]. In addition, Clinpro WV has previously been shown to be capable of penetrating into the dentin tubules up to 13 µm, forming a layer on their walls, thus reducing their diameter [11]. Nevertheless, it has also previously been observed that although the amounts of fluoride released by Clinpro WV into a lactic acid solution was four times higher than that released by Duraphat within 24 h of exposure, there were no significant differences in calcium release between the two varnishes [21]. In another study, the calcium release of two varnishes did not differ for a period of up to 72 h [22]. This could explain the similar behavior of these varnishes in the present investigation, as the ability to form CaF_2-like deposits is known to be limited by the availability of calcium [23]. Our results were in agreement with a past study conducted by our group, in which neither Duraphat nor Clinpro WV were able to sustain tubule occlusion post-cycling [17].

Clinpro XT is a resin-modified and light cured ionomer varnish that contains fluoride, calcium and phosphate [12]. Its ability to seal the dentinal tubules is related to its chemical adhesion to the dentin, in addition to the release of the above-mentioned ions. Clinpro XT was observed to release more fluoride into saliva in a period of up to 6 months than two other commercially available varnishes [24]. The authors hypothesized that this extended fluoride release was due to the chemical bond between this varnish and the tooth, which is related to its resin modified glass ionomer technology. In a previous investigation by our group, Clinpro XT varnish was able to promote tubule occlusion post- application and also to maintain this effect after being submitted to erosive-abrasive cycling similar to the type used in the present study [17]. In this previous study, however, tubule occlusion was only analyzed by ESEM, and in some specimens it was possible to observe complete detachment of the varnish from the dentin surface. A similar finding was observed in the present study, in addition to partial debonding of the varnish in other specimens, which could explain their higher ODT values in the post-cycling period. Considering the small area used in both tests, it is possible to infer that in many spots where there was absence of varnish, were evaluated. Another possibility was that the application of pulpal pressure during the permeability analysis helped to remove the treatment from the surface, as this effect was not simulated in our previous study [17]. Another in vitro investigation found that Clinpro XT was able to promote tubule occlusion, and this result was persistent without increase in dentin permeability after three erosive challenges performed with different beverages [25]. Considering this result, we could suggest that in the present study, the presence of a higher number of erosive episodes, in addition to the action of toothbrushing, could have promoted a more vigorous removal of the Clinpro XT varnish from the surface. We opted to use this type of model in attempt to simulate individuals with high consumption of acidic beverages and who brush their teeth twice a day [17]. It should, however, be mentioned that clinically, Clinpro XT varnish had the ability to reduce the pain in DH for a period of up to 6 months of evaluation [26].

Teethmate Desensitizer is a calcium/phosphate-based paste made up of a powder that consists of tetracalcium phosphate (TTCP) and dicalcium phosphate anhydrous (DCPA), which is mixed with water, resulting in a layer similar to the hydroxyapatite [27], which can seal the dentinal tubules and fill enamel cracks. The effect of Teethmate in the hydraulic conductance of dentin has been evaluated in a previous

study, in which it reduced dentin permeability by 92% immediately after application [28]. Accordingly, in the present investigation, it was able to reduce dentin permeability immediately after application in a manner similar to that of varnishes. In the post-treatment micrographs, the number of ODTs decreased significantly, and although the material did not cover the dentin surface in the same way as the varnishes did, it seemed to have induced the penetration of deposits within the dentin tubules. This was in agreement with a previous investigation [28]. However, as occurred with the other treatments, in the post-cycling period, its permeability did not significantly differ from that of the control. To the best of our knowledge, there are no studies evaluating the resistance of this material to chemical and mechanical challenges. From the micrographs, it would appear that the deposits created by the material were removed post-cycling. Clinically, an immediate and long lasting desensitizing action of Teethmate was found throughout a period of 6-months of observation [29].

Desensibilize NanoP is a desensitizing agent in a vehicle in the form of paste that contains 9000 ppm of fluoride and potassium nitrate, in addition to a compound of calcium phosphate. According to the manufacturer, its nanometric size and crystalline form enables it to penetrate into the dentinal tubules and conserve a certain stability and resistance to acid challenges. The calcium phosphate nanoparticles were observed to exhibit properties that were twice as good as those of the traditional calcium phosphate compositions [30]. In agreement with a previous in vitro study [17], NanoP was able to reduce the number of ODTs after treatment, but not after erosive-abrasive cycling. In the post-cycling micrographs, the number of ODT increased, but not in a manner equal to that of the post-EDTA treatment. In the post-cycling micrographs, there seemed to be deposits of the material on the dentin surface, and tubules with reduced diameter, possibly due to the remineralization action of fluoride and calcium phosphate, but this was not capable of significantly reducing the permeability of dentin. In a recent clinical study, this material was able to reduce the pain in DH for a period of up to 3 months of evaluation [31]. Nevertheless, in this case, the role of potassium nitrate in inactivating the intradental nerves should also be considered [31].

Post-treatment, Nupro prophylaxis paste containing 15% of calcium sodium phosphosilicate, an amorphous inorganic material based on a class of "bioglass" (known as NovaMin®), significantly decreased dentin permeability; however, it presented higher Lp% values when compared with the other groups. In addition, in the ESEM evaluation, the number of ODTs it produced did not differ significantly from the control value. As discussed earlier, this could also be related to the more heterogeneous deposits created by this product. Furthermore, it could also be suggested that in ESEM analysis, the performance of the material was reduced because Nupro requires an exposure to an aqueous media to react and form a mineralized layer containing calcium, phosphate and silicate that obliterates the dentinal tubules [32–35]. Since the application of the paste was made in vitro, its effect was lower than that shown in the permeability assessment, in which the material that penetrated into the dentin tubules post-application reacted with the distilled water of the permeability test machine, thus the paste presented a significant reduction in the Lp% post-treatment. Supporting this hypothesis, a previous in vitro study applied the paste as a slurry with saliva, and found a significant reduction in the %Lp after treatment, and also after erosion-abrasion cycling [15]. In an in situ investigation, Nupro showed capacity to occlude the dentinal tubules and to resist to chemical challenges [36]. Clinically, an immediate reduction in pain was observed with this product, which persisted during a one-week [37] and one-month follow-up [38]. Nevertheless, in the present investigation, as was the case with the other products in the post-cycling period, Nupro was not capable of maintaining low permeability.

For the control group, as expected, in the post-treatment period there was no reduction in dentin permeability and in the number of ODTs. However, in the post-cycling period, as shown in the ESEM assessment, the control presented a significant reduction in the number of ODTs. As observed in the ESEM micrographs, this could be related to the smear layer created by toothbrushing, which may have contributed to occluding some dentin tubules, as has also been seen in other studies [15,17,39]. This effect of toothbrushing could also explain the lack of difference between the experimental post-treatment and post-cycling time intervals for some groups, such as Clinpro XT, Nupro, Nano P and Teethmate, as shown in the ESEM assessment. One limitation of the study was the use of a toothpaste containing sodium monofluorophosphate in its composition. Since this compound requires enzymatic breakdown from saliva to release fluoride [40], a significant fluoride effect from the toothpaste in promoting tubule occlusion is not expected.

The in vitro model used in the present investigation considers the assumption that dentin tubule occlusion is associated with a reduction of the pain from DH in the clinical setting [41,42]. However, clinical data confirming the effectiveness of the treatments is warrant, as in the clinical scenario there are other factors implicated in the reduction of the pain in DH, such as the modification of pulpal nerve excitation, an effect observed with some agents, as potassium nitrate present at Desensibilize NanoP cited earlier. The findings of this study support the effectiveness of the calcium phosphate compounds to occlude the dentin tubules after application, which could be associated to an immediate pain relief at the clinic. Nevertheless, long-term clinical studies are needed to evaluate the resistance of these products to the day-to-day challenges, as none of the products was able to maintain tubule occlusion under a simulation of individuals with high frequency of acidic beverage consumption.

5. Conclusion

Under the conditions and limitations of this *in vitro* investigation, it could be concluded that all treatments were efficient in reducing dentin permeability and the number of open dentin tubules after their application, with Nupro only being effective in the permeability analysis. However, none of the products was able to resist the chemical and mechanical challenges.

Conflict of interest

None

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