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Assessment of the Ability of Desensitizing and Conventional Mouth Rinses to Promote Dentin Tubule Occlusion

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Clinical Relevance

Mouth rinses are an alternative vehicle for the delivery of desensitizing agents in a daily regimen. However, no significant tubule occlusion indicates more evidence is needed to establish their effectiveness against dentin hypersensitivity.

SUMMARY

This study aimed to evaluate the effect of desensitizing and conventional mouth rinses on dentin tubule occlusion. Dentin hypersensitivity was simulated by EDTA application for five minutes. The specimens were randomly allocated into the following groups: desensitizing mouth rinses (Colgate Sensitive, Elmex Sensitive Professional, Listerine Advanced Defense Sensitive, Sensodyne Cool Mint); conventional mouth rinses (Colgate Plax, Elmex Caries Protection, Listerine Anticaries, Sensodyne Pronamel); a negative control (C-: distilled water); and Clinpro XT Varnish was the positive control (C+). Subsequently, the specimens were submitted to an erosive or abrasive challenge (performed separately) and to an erosive/abrasive cycling for five days (n=10 for each challenge). After treatment, challenges, and cycling, the specimens were analyzed in an environmental scanning electron microscope to verify the number of open dentin tubules (ODTs), counted by using

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Image J software (National Institutes of Health, Bethesda, MD, USA). Data were analyzed by the Kruskal-Wallis, Friedman and Dunn tests, with Bonferroni correction (α=0.05). Groups did not differ at baseline (p>0.05). At the post-treatment, erosion and abrasion stages, C+ was the only group that showed a reduction in ODTs compared to C- (p<0.05). In the other groups, numbers did not differ significantly from C- (p>0.05). After cycling, none of the groups exhibited significant reduction in ODTs other than C- (p>0.05); however, C+, Listerine Anticaries, and Colgate Plax had a lower number of ODTs than Listerine Sensitive and Sensodyne Pronamel. No mouth rinse was able to promote significant occlusion of the dentin tubules after treatment and the challenges. C+ was the only product that effectively promoted tubular occlusion, but this effect did not withstand several erosive and abrasive challenges.

INTRODUCTION

Dentin hypersensitivity (DH) is characterized by a short and sharp pain that occurs in response to external stimuli acting on an area of exposed dentin surface, with open and patent tubules.[1] Erosive tooth wear (ETW) can often result in dentin exposure, and the impact of erosive acids and toothbrushing abrasion can contribute to the opening and enlargement of the dentinal tubules, thus leading to DH.[2]

The most widely accepted theory to explain the pain caused by DH is the hydrodynamic theory, which explains that any external stimuli acting on the dentin surface can promote movement of the dentin fluid, located inside the tubules. This would then stimulate the mechanoreceptors from the pulp, resulting in pain.[3] In order to prevent the movement of this fluid, one of the main strategies relies on sealing of the dentinal tubules.[4] Products intended for use at home, such as toothpastes and rinses, or in-office products such as gels, varnishes, modified glass ionomer cements and adhesives are indicated[5]; however, no standard product for DH treatment has yet been determined.[6]

Fluoridated products are widely used for controlling DH. These products are capable of promoting obliteration of the dentin tubules by the formation and deposition of CaF_2-like precipitates.[7] However, this protection may be clinically limited, especially in subjects exposed to a high frequency of erosive episodes.[4] Products that are regularly applied, such as toothpastes and mouth rinses, could be a way to overcome this limitation; nevertheless, these products have only a low to moderate fluoride concentration, thus only low amounts of CaF_2-like precipitates are expected.[8] In addition, fluoride does not sustain much substantivity in the oral cavity when delivered in these vehicles.[9]

In view of this, desensitizing agents have been incorporated into some commercially available mouth rinse formulations. Examples of these agents are: arginine with the copolymer polyvinylmethyl ether-maleic acid (PVM/MA),[10] potassium oxalates[11] and potassium nitrate.[12] The advantages of using mouth rinses over toothpastes would be the absence of the mechanical action of toothbrushing, which can influence the efficacy of the active ingredient.[13] Additionally, desensitizing mouth rinses are isotonic,[14] and do not include a tactile stimulation of pain.[15] Moreover, the ability of the product to promote tubule occlusion is believed to increase when the product is in liquid form.[16]

At present, there are many desensitizing mouth rinses available in the market. However, according to the best of the authors' knowledge, there are no studies that have systematically compared the effect of these products with those products that have similar formulations but do not contain desensitizing agents. There is also very little information available about the ability of these products to maintain tubule occlusion when exposed to the challenges present in the oral cavity. Therefore, the aim of the present study was to investigate the efficacy of different desensitizing mouth rinses in promoting tubule occlusion, as well as their resistance to erosive/abrasive challenges.

The null hypotheses tested were: (1) There would be no differences between the mouth rinses and the negative control group (distilled water) regarding their ability to promote tubule occlusion immediately after treatment; and (2) There would be no differences in the tubular occlusion promoted by the mouth rinses and the negative control group after the erosive and abrasive challenges (performed separately) and also not after being submitted to erosive-abrasive cycling.

METHODS AND MATERIALS

Experimental Design

The mouth rinses were tested using an erosive or abrasive challenge, performed separately (Experiment 1) or using an erosion-abrasion cycling model (Experiment 2). In addition to the desensitizing mouth rinses, the conventional versions of each brand, without desensitizing agents, were also tested for the purpose of comparison. The study followed a completely randomized design with two experimental factors for Experiment 1: (1) Mouth rinses, at 10 levels: desensitizing mouth rinses (Colgate Sensitive,

Elmex Sensitive Professional, Listerine Advanced Defense Sensitive, and Sensodyne Cool Mint [Table 1]); conventional mouth rinses (Colgate Plax, Elmex Caries Protection, Listerine Anticaries, and Sensodyne Pronamel [Table 1]); negative control (C- : distilled water), and positive control (C+ : Clinpro XT Varnish [Table 1]); (2) Experimental time, at three levels for the erosive and abrasive challenges (baseline, post treatment and post challenges). For Experiment 2, the cycling model, there was only one experimental factor, mouth rinses, at 10 levels, as mentioned previously. The experimental units were specimens of human dentin (n=10 for each challenge). The response variable was the number of opened dentinal tubules (ODTs). Tubule counting was performed with Image J software (National Institutes of Health, Bethesda, MD, USA) on environmental scanning electron microscopy images. Dentin morphology was also qualitatively evaluated in the micrographs, at each experimental time interval.

Specimen Preparation

Three hundred (300) sound human third molars were collected after the approval of the Local Ethics Committee (CAAE number: 89906318.7.0000.0075). From the roots of the teeth, dentin slabs (3 mm × 3 mm × 2 mm) were obtained using an automatic cutting machine (Isomet 1000, Buehler Ltd, Lake Bluff, IL, USA). To standardize the test area, abrasive papers with decreasing granulations were used (800-, 1200- and 4000-grit; Struers Inc, Ballerup, Denmark), in a polishing machine (Tegramin, Struers Inc.) under constant cooling. After each paper and at the end of the polishing sequence, the specimens were sonicated with distilled water for 3 minutes to remove any debris.

Opening of the Dentin Tubules

To simulate a hypersensitive dentin, the specimens were immersed in EDTA solution (17%, pH=7.4) for 5 minutes to open the dentin tubules and to remove the smear layer. After this step, the specimens were abundantly rinsed with deionized water.[17] The initial number of ODTs of the specimens were analyzed and considered as the reference for the change in dentin tubular occlusion after each experimental time interval.

Experiment 1—For each challenge, the specimens were stratified into 10 experimental groups (n=10 per group) according to their number of open dentin tubules. Then, one-way analysis of variance (ANOVA) was conducted to certify that the number of open tubules did not differ significantly among the groups (p>0.05). Treatments were performed according to the manufacturer's instructions, as described in

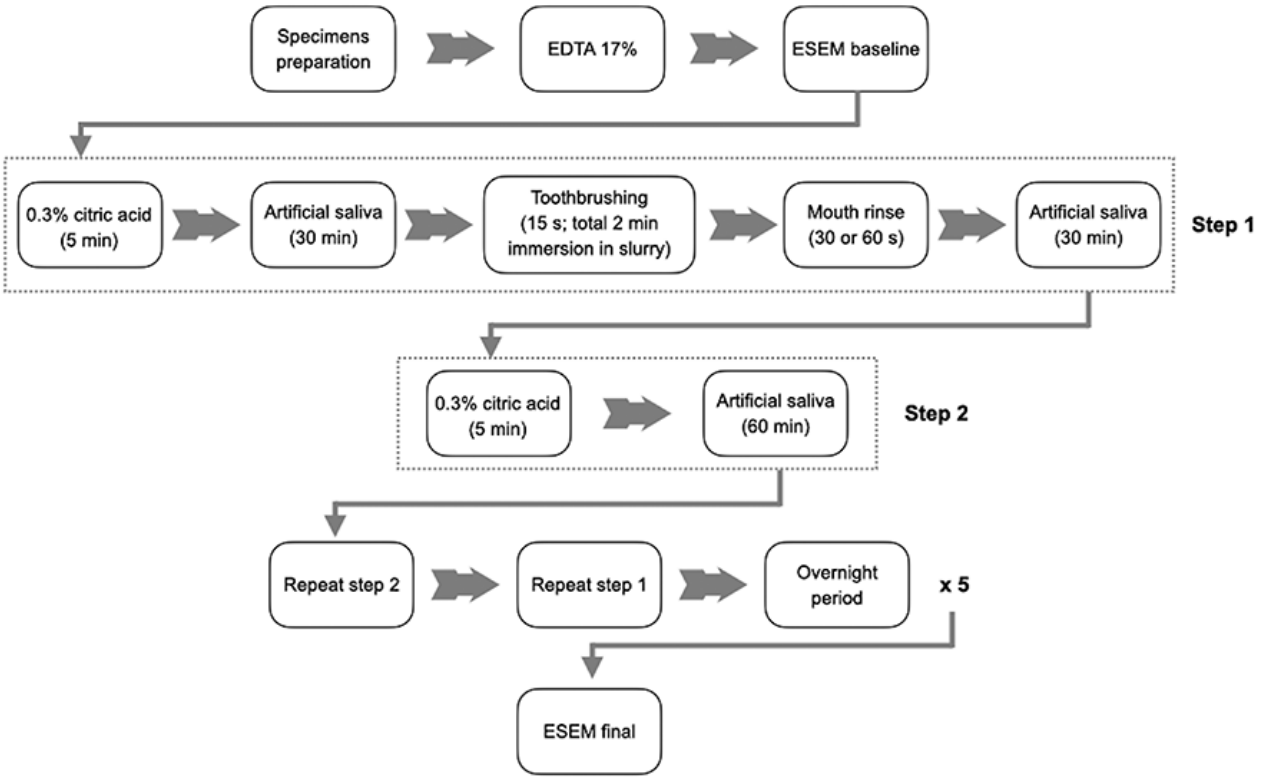

Figure 1. *Flow diagram schematizing the experiment 2. Abbreviations: ESEM, environmental scanning electron microscopy.*

Table 1: *Details of the Groups: Products, Manufacturers, Composition, Protocol of Application, pH, and Desensitizing Mechanism*

Group	Product	Manufacturer	Main Composition
Desensitizing mouth rinses			
Colgate Sensitive	Colgate Sensitive Pró-Alívio	Colgate Palmolive Industrial Ltda, São Paulo, SP, Brazil	Arginine (0.8%), Aqua, Glycerin, Sorbitol, Propylene Glycol, Tetrapotassium Pyrophosphate, PEG-40, Hydrogenated Castor Oil, PVM/MA Copolymer, Polysorbate 20, Tetrasodium Pyrophosphate, Aroma, Benzyl Alcohol, Sodium Fluoride (NaF) (225 ppm F^-), Menthol, Sodium Saccharin, Citric Acid, Methylisothiazolinone, CI 42051, CI 17200
Elmex Sensitive	elmex SENSITIVE Professional	GABA, Colgate Palmolive Manufacturing, Swidnica, Poland	Arginine (0.8%), Aqua, Glycerin, Sorbitol, Propylene Glycol, Disodium Pyrophosphate, PEG-40 Hydrogenated Castor Oil, PVM/MA Copolymer, Tetrapotassium Pyrophosphate, Sodium Levulinate, Olaflur (AmF) (125 ppm F^-), Aroma, Potassium Hydroxide, Sodium Saccharin, Sodium Fluoride (NaF) (125 ppm F^-), C.I. 19140, C.I. 42051
Listerine Sensitive	Listerine Advanced Defense Sensitive	Johnson & Johnson Limited, Maidenhead, Berkshire, United Kingdom	Dipotassium Oxalate (1.4%), Aqua, Sorbitol, Propylene Glycol, Phosphoric Acid, Aroma, Poloxamer 407, Sodium Benzoate, Sodium Methyl Cocoyl Taurate, Sodium Lauryl Sulfate, Sucralose, Sodium Saccharin, Sodium Fluoride (NaF) (220 ppm F^-)
Sensodyne Cool Mint	Sensodyne Cool Mint	Glaxosmithkline Brasil Ltda, Jacarepaguá, RJ, Brazil	Aqua, Glycerin, Sorbitol, Potassium Nitrate, PEG-60 Hydrogenated Castor Oil, Poloxamer 407, Sodium Benzoate, Aroma, Disodium Phosphate, Methylparaben, Propylparaben, Sodium Phosphate, Sodium Fluoride, Sodium Saccharin, CI 42090. Contains 3% w/w Potassium Nitrate and 0.048% w/w Sodium Fluoride (NaF) (217 ppm F^-)
Conventional mouth rinses			
Colgate Plax	Colgate Plax Soft Mint	Colgate Palmolive Industrial Ltda, São Paulo, SP, Brazil	Aqua, Glycerin, Propylene Glycol, Sorbitol, Poloxamer 407, Aroma, Cetylpyridinium Chloride, Potassium Sorbate, Sodium Fluoride, Sodium Saccharin, Menthol, CI 42051, Contains: Sodium Fluoride (NaF) 0.05% (225 ppm F^-)
Elmex Caries Protection	elmex Kariesschutz	GABA, Colgate Palmolive Manufacturing, Swidnica, Poland	Aqua, Propylene Glycol, PEG-40 Hydrogenated Castor Oil, Olaflur, Glycerin, Sodium Benzoate, Levulinic Acid, Sodium Levulinate, Aroma, Saccharin, Sodium Fluoride, Sodium Anisate. Contains: Olaflur (AmF) and Sodium Fluoride (NaF) total fluoride content: 250 ppm. Aroma (> 100 PPM): Anethole, Menthol
Listerine Anticaries	Listerine Anticaries	Johnson & Johnson Brazil. Industry and Trade of Health Products Ltda, São Paulo, SP, Brazil	Aqua, Sorbitol, Propylene Glycol, Poloxamer 407, Sodium Lauryl Sulfate, Benzoic Acid, Aroma (Benzyl Alcohol, d-limonene), Eucalyptol, Methyl Salicylate, Thymol, Sodium Saccharin, Sodium Fluoride (NaF) (220 ppm F^-), Sodium Benzoate, Sucralose, Menthol, CI 47005, CI 42053

Table 1: *Details of the Groups: Products, Manufacturers, Composition, Protocol of Application, pH, and Desensitizing Mechanism (ext.)*

Protocol (Immersion Time)	pH	Desensitizing Mechanism
60 s	8.38	Arginine is a positively charged amino acid, which binds to the dentin, which has a negative charge. Under basic pH values, there is the deposition of arginine, calcium, phosphate, and carbonate on dentin and within dentinal tubules. The copolymer PVM/MA has the ability to adhere to the dentin by Van der Waals forces and by hydrogen bonding with surface proteins, acting by maintaining the active principles adhesively retained on the oral tissues. Pyrophosphate stabilizes the complex in the solution and has the ability to deposit on the dentin surface
30 s	6.23	
60 s	4.24	Its mode of action relies on the formation of calcium oxalate crystals on the dentin surface and inside dentinal tubules
60 s	6.65	The mode of action for potassium desensitization is by increasing the intratubular K^+ concentration, making the intradental nerves less excitable to the stimuli through depolarization of the nerve fiber membrane. Initially, this increase in K^+ content causes an increase in the number of action potentials after initial depolarization. The layered fiber cannot repolarize due to the maintenance of high levels of extracellular K^+ and, consequently, a sustained state of depolarization (axonal accommodation) occurs. It was shown that these products can also have tubule occlusion properties, which may be due to the action of other ingredients in the formulation (such as silica and sodium fluoride)
60 s	5.09	
30 s	4.45	
60 s	4.18	

Table 1: *Details of the Groups: Products, Manufacturers, Composition, Protocol of Application, pH, and Desensitizing Mechanism*

Group	Product	Manufacturer	Main Composition
Sensodyne Pronamel	Pronamel Daily Mouthwash	GlaxoSmithKline group of companies, Brentford, Middlesex, United Kingdom	Aqua, Glycerin, Sorbitol, Poloxamer 338, PEG-60 Hydrogenated Castor Oil, VP/VA Copolymer, Potassium nitrate, Sodium benzoate, Cellulose Gum, Aroma, Sodium fluoride, Methylparaben, Propylparaben, Cetylpyridinium Chloride, Sodium Saccharin, Xanthan Gum, Disodium Phosphate, Sodium Phosphate, CI 42090, Sodium Fluoride (NaF) 0.1% w/w (450 ppm F^-)
Control			
Negative control C-	Distilled water	—	Distilled water
Positive control C+	ClinproXT Varnish	3M ESPE, Sumaré, SP, Brazil.	Part A: glass particles of silanized fluoro-aluminosilicate, HEMA, water, BIS-GMA, and silanized silica Part B: copolymer of polyalkenoic acid, water, HEMA and calcium glycerophosphate

Table 1. The specimens were treated according to the experimental groups, immediately followed by 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 HCI)[18] for 60 minutes and then submitted to the challenge (erosion or abrasion). The challenges were performed separately in order to assess the individual effect of each type of challenge. In addition to the desensitizing mouth rinses, the conventional versions of each brand, without desensitizing agents, were also tested for the purposes of comparison.

The erosive challenge, equivalent to one day of acid exposure time of the five-day erosion-abrasion cycling, consisted of 20 minutes immersion in citric acid (0.3%, pH~2.6). As regards the specimens submitted to the abrasive challenge, they were brushed in an automatic brushing machine (Biopdi, Sao Paulo, Brazil), with soft brushes (Oral-B Indicator Plus, Procter & Gamble, São Paulo, Brazil) (150 g, 90 strokes) for 30 seconds, which was also intended to simulate one day of brushing time of the cycling model. Brushing was performed with a slurry of conventional toothpaste, without desensitizing agents (Elmex Anticáries, Colgate Palmolive, Brazil, amine fluoride, 1400 ppm F^-), and artificial saliva, in a ratio of 1 part of dentifrice to 3 parts of artificial saliva. Total time of exposure to the slurry was 4 minutes.

Experiment 2—In this phase, new human dentin specimens with open dentin tubules were stratified into the previously described groups. One-way ANOVA was conducted to assure that the groups did not differ significantly among them ($p>0.05$). Then, the specimens were submitted to an erosion-abrasion cycling model. The specimens were immersed in 0.3% citric acid solution (pH~2.6) for 5 minutes, without agitation, followed by immersion in artificial saliva for 60 minutes. This procedure was repeated 4 time per day for five days.[19] Toothbrushing was performed twice a day in the middle of the first and last periods of saliva exposure. The specimens were brushed as previously described for 15 seconds (150 g, 45 strokes). The slurry was prepared prior to each abrasive challenge and the specimens were exposed to the slurry for 2 minutes. Then, the specimens were exposed to the commercial mouth rinses for 30 or 60 seconds, depending on the manufacture's instruction. After each erosive and abrasive challenge, the specimens were rinsed with distilled water and gently dried with absorbent paper. At the end of each cycling day, the specimens were kept in a humid chamber during the overnight period. The citric acid solution and the mouth rinses were replaced after each use and artificial saliva was renewed once a day (Figure 1).

Table 1: *Details of the Groups: Products, Manufacturers, Composition, Protocol of Application, pH, and Desensitizing Mechanism (ext.)*

Protocol (Immersion Time)	pH	Desensitizing Mechanism
60 s	6.29	
60 s	6.93	
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	—	

Environmental Scanning Electron Microscopy Evaluation (ESEM)

After EDTA application, all specimens were analyzed by environmental scanning electron microscopy (ESEM) (Hitachi TM3000, Hitachi, Tokyo, Japan) to make a qualitative evaluation and quantitative calculation of the number of open dentin tubules. Representative micrographs were taken at magnifications of 2000×, by using Analy observation conditions, at the center, northwest, and southeast of each specimen. No sample preparation was required. For the erosive and abrasive challenges, the specimens were re-evaluated after the treatments and the challenges. For the erosive-abrasive cycling, they were re-evaluated post-cycling. In the qualitative assessment, the micrographs were evaluated and checked for patency and occlusion of the dentin tubules. The quantitative assessments were made using an image analysis software program, ImageJ (NIH).[20] An average of the open dentin tubules counted in the three images taken for each specimen were considered in the statistical analysis.

Statistical Analyses

Normality and homoscedasticity of data were checked with Shapiro-Wilks and Brown-Forsythe tests, respectively. Since ODT data did not follow a normal distribution, Kruskal-Wallis, Friedman, and Dunn tests were performed, with Bonferroni corrections for multiple comparisons, considering a significance level of 5%. The analyses were performed using the software SigmaPlot 13 (Systat Software Inc, San Jose, CA, USA).

RESULTS

Erosive Challenge

The medians and interquartile intervals of ODTs for all groups at baseline, post-treatment, and post-erosion are described in Table 2. At baseline, the groups did not differ significantly ($p>0.05$). The varnish C+ produced the lowest statistically significant number of ODTs post-treatment, in comparison with all the other groups ($p<0.05$), except for the Colgate Plax Group ($p=0.094$). The other groups did not differ significantly from each other or from C- ($p>0.05$).

After erosion, C+ continued to show a lower number of ODTs, without significant differences, from Sensodyne Pronamel ($p=0.08$), Colgate Plax ($p=0.225$), and Listerine Sensitive ($p=0.606$), which showed no significant differences from the other groups, including C- ($p>0.05$).

There were no significant differences among the experimental times for Colgate Sensitive, Elmex

Table 2: *Median and Interquartile Intervals for the Number of Open Dentin Tubules (ODTs) for All Groups at Baseline, After Treatment and After Erosion*[a]

Groups	Baseline		Post Treatment		Post Erosion	
C-	119.5 (96.7-144.5)	Aa	107.0 (90-135.7)	Ab	134.5 (87.7-164.5)	Aab
Colgate Sensitive	118.0 (95.0-142.2)	Aa	158.0 (110.7-208.2)	Aa	172.5 (143.7-214)	Aa
Listerine Anticaries	119.0 (92.2-142.0)	Aab	114.0 (98.2-132.7)	Ab	149.5 (102-175.5)	Aa
Elmex Sensitive	117.5 (99.0-136.0)	Aa	112.5 (99-124.2)	Aa	128.5 (118-149.7)	Aa
Elmex Caries Protection	119.5 (96.0-143.7)	Aab	112.5 (94.5-140)	Ab	147.0 (135-184.7)	Aa
Sensodyne Pronamel	117.5 (93.2-138.0)	Aab	112.0 (92.2-129.5)	Ab	123.5 (98.5-141.5)	ABa
Sensodyne Cool Mint	116.5 (98.0-134.7)	Aab	111.0 (85.5-128.5)	Ab	136.0 (113-155.5)	Aa
Colgate Plax	114.0 (91.0-137.0)	Aa	97.0 (83.7-134)	ABa	107.5 (88.7-141)	ABa
Listerine Sensitive	115.5 (97.0-133.0)	Aa	85.0 (65.7-131)	Ab	95.5 (77.5-139.7)	ABab
C+	119.0 (96.0-143.0)	Aa	0.0 (0.0-0.0)	Bb	0.0 (0.0-0.2)	Bb

Abbreviations: C-, negative control; C+, positive control.

[a]*In columns, different capital letters show significant differences among groups at each experimental time interval (p<0.05). In rows, different lowercase letters show significant differences between the experimental time intervals for each group (p<0.05).*

Sensitive, and Colgate Plax (p=0.150, p=0.071, and p=0.179, respectively). For C- and Listerine Sensitive, the number of ODTs at baseline was significantly higher than post-treatment (p=0.022 and p=0.042, respectively), but both experimental time intervals did not differ significantly from post-erosion (p>0.05). For Listerine Anticaries, Elmex Caries Protection, Sensodyne Pronamel, and Sensodyne Cool Mint, the number of ODT post-treatment was significantly lower than post-erosion (p<0.05), and both of these experimental times did not differ significantly from baseline (p>0.05). For C+, the number of ODT post-treatment and post-erosion did not differ significantly (p>0.05), and both experimental time intervals showed lower ODTs than baseline (p<0.01).

Abrasive Challenge

The medians and interquartile intervals of ODTs for all groups post-treatment and post-abrasion are shown in Table 3. At baseline, the groups did not differ significantly (p>0.05). Post-treatment, C+ was observed to be the only group that showed significant reduction in the number of ODTs than C- (p=0.031) and the other groups (p<0.05).

After abrasion, C+ had the lowest number of ODTs and the only group that differed significantly from C- was C+ (p=0.041). The other groups did not differ from each other (p>0.05).

For groups C-, Colgate Plax, Colgate Sensitive, Listerine Sensitive, Listerine Anticaries, and Sensodyne Cool Mint, the number of ODTs post-treatment was significantly lower than post-abrasion (p<0.05); however, both experimental time intervals did not differ significantly from baseline (p>0.05). For the group Elmex Sensitive, the number of ODTs was significantly higher post-abrasion than post-baseline (p=0.011) and both experimental time intervals did not differ significantly from post-treatment (p=0.791 and p=0.221, respectively). For Elmex Caries Protection, the number of ODTs post-treatment was significantly lower than baseline (p=0.042) and post-abrasion (p=0.004), which in turn did not differ significantly from each other (p>0.05). For C+, the number of ODTs of post-treatment and post-abrasion did not differ (p>0.05), but it was significantly lower than baseline (p<0.01). For Sensodyne Pronamel, there were no significant differences among experimental time intervals (p>0.05).

Erosive-abrasive Cycling

The medians and interquartile intervals of ODTs for all groups after cycling are described in Table 4. C+ presented the lowest number of ODTs with significant differences from Listerine Sensitive (p=0.001). The other groups did not differ significantly from Group C+ (p>0.05).

ESEM Evaluation

In Figures 2 and 3, row A shows the micrographs of the groups after 17% EDTA conditioning. For all groups, there were open dentin tubules, without the presence of a smear layer or other particles and slight demineralization of the peritubular dentin. The intertubular dentin had a polished appearance. Row B shows the micrographs taken after treatment. For all

Table 3: *Median and Interquartile Intervals of the Numbers of Open Dentin Tubules (ODTs) for All Groups at Baseline, After Treatment and After Abrasion*[a]

Groups	Baseline		Post treatment		Post abrasion	
C-	118.0 (94.7-139.2)	Aab	105.0 (83.7-124.0)	Ab	155.0 (122.2-221)	Aa
Colgate Sensitive	117.5 (99.2-136.2)	Aab	111.5 (86.2-127.5)	Ab	181.5 (125.5-206.2)	Aa
Listerine Anticaries	116.0 (98.0-133.7)	Aab	101.0 (90.0-110.0)	Ab	174.5 (134.2-218.7)	Aa
Elmex Sensitive	115.5 (91.5-137.0)	Ab	104.5 (103.7-224.0)	Aab	173.5 (155.5-189.2)	Aa
Elmex Caries Protection	117.5 (94.0-138.5)	Aa	114.5 (80.2-129.7)	Ab	159.5 (121-206.2)	Aa
Sensodyne Pronamel	116.5 (98.2-135.0)	Aa	123.0 (99.5-143.0)	Aa	140.0 (120.7-168.0)	Aa
Sensodyne Cool Mint	118.5 (95.0-141.0)	Aab	92.5 (63.2-125.7)	Ab	225.5 (190.0-260.7)	Aa
Colgate Plax	117.5 (99.2-136.2)	Aab	112.0 (100.5-125.7)	Ab	233.0 (151.2-296.2)	Aa
Listerine Sensitive	117.0 (92.7-138.0)	Aab	108.0 (48-136.7)	Ab	182.0 (138.0-195.2)	Aa
C+	119.0 (96.0-143.0)	Aa	0.0 (0.0-0.0)	Bb	0.0 (0.0-1.2)	Bb

Abbreviations: C-, negative control; C+, positive control.
[a]*In columns, different capital letters show significant differences among groups within experimental time intervals ($p<0.05$). In rows, different lowercase letters show significant differences between the experimental times within groups ($p<0.05$).*

groups, there were visible open dentin tubules, except in C+, which showed the dentin surface covered by the vitreous particles of the material. Row C shows the micrographs of the groups after the erosive challenge. After acid etching, the smear layer was removed, and the dentin tubules were open. The peritubular dentin was completely demineralized. The only group that presented tubular occlusion was C+ with a large number of particles on the surface, covering the tubular lumina. Row D shows the micrographs of the groups after the abrasive challenge. For all groups, some small particles could be seen at the orifices of the dentin tubules, without noteworthy obstruction of the respective lumina. C+ was the only group that had a material covering the opened dentin tubules. Row E shows the micrographs of the groups after cycling. In all of them, except C+, a visibly worn surface with opened dentin tubules could be observed. A larger number of particles were visible at the tubule orifices, without completely obstructing them. C+ samples continued to show the vitreous-like particles of the material with some sparse dentin tubules visible.

Table 4: *Median and Interquartile Intervals for the Number of Open Dentin Tubules (ODTs) for All Groups After Cycling*[a]

Groups	After Cycling
C-	84.0 (71.7/125.2) AB
Listerine Sensitive	126.0 (102.2/159.2) A
Sensodyne Pronamel	107.0 (56.5/146.7) AB
Elmex Caries Protection	101.0 (96.5/106.2) AB
Sensodyne Cool Mint	95.0 (84.5/97.5) AB
Colgate Sensitive	89.0 (74/111) AB
Elmex Sensitive	84.5 (67.2/112.2) AB
Colgate Plax	78.5 (55.5/94) B
Listerine Anticaries	78.5 (62.5/91.25) B
C+	60.0 (46.5/81.75) B

Abbreviations: C-, negative control; C+. positive control.
[a]*Different letters show significant difference among Groups ($p<0.05$).*

DISCUSSION

In the present study, we endeavored to compare the effect of desensitizing mouth rinses with their conventional versions without desensitizing agents on dentin tubule occlusion. The resistance of the effect promoted by the mouth rinses to erosive and abrasive challenges, performed separately, and their ability to promote tubule occlusion under cyclic erosion-abrasion conditions was also tested. The erosive challenge consisted of uninterrupted immersion of the specimens in 0.3% citric acid (pH 2.6) for 20 minutes, simulating one day of acid consumption of an individual with high risk for ETW.[18] For the abrasive challenge, a brushing period of 30 seconds was carried out. The 30-second time sequence was made on the assumption that each individual brushes her/his teeth for a total of two minutes, two times daily. Therefore, each quadrant would be brushed for 15 seconds each time, for a total of 30 seconds a day.[4] Testing the mouth rinses under cyclic erosion-abrasion conditions is important because

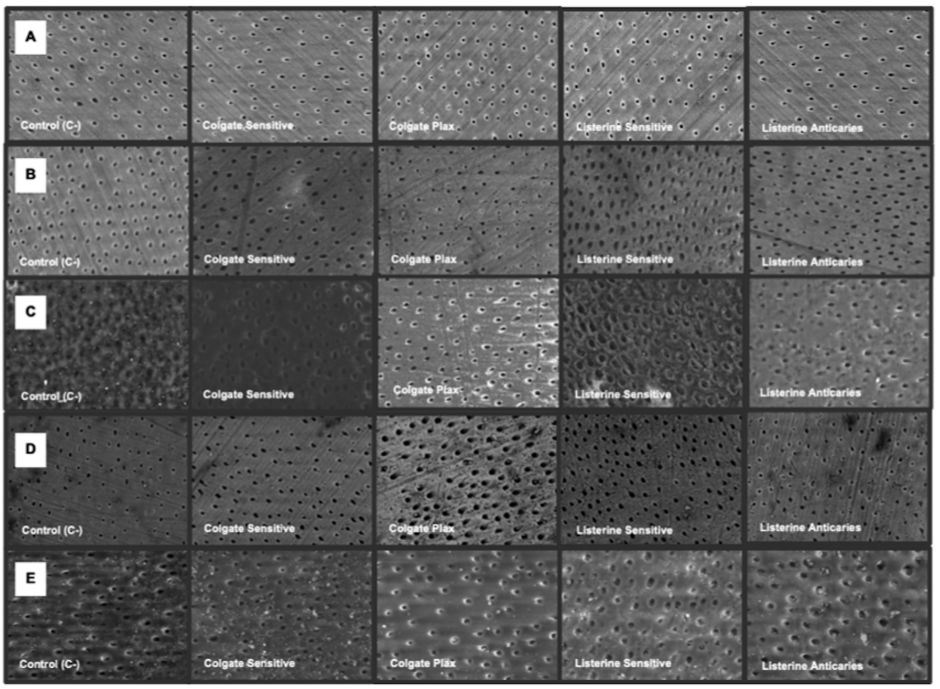

Figure 2. *(A) Dentin surface after 17% EDTA. (B) Dentin surface after treatment. (C) Dentin surface after erosion challenge showing a visibly worn treatment layer with dentin exposure. (D) Dentin surface after abrasion challenge showing a visibly worn treatment layer with dentin exposure. (E) Dentin surface after erosion/abrasion cycling showing a visibly worn treatment layer with dentin exposure. Control (C-) - Distilled water. Colgate Sensitive. Colgate Plax. Listerine Sensitive. Listerine Anticaries.*

this allows the possible cumulative effect of the agents to be evaluated, as well as their ability to withstand frequent chemical and mechanical challenges. Under all the conditions used, none of the mouth rinses tested were able to promote significant tubule occlusion when compared to the negative control after treatment and challenges, thus the two null hypotheses of this study were accepted. Nevertheless, it should be noted that, in Experiment 1, some groups presented some degree of tubule occlusion when comparing the post-treatment time interval with the baseline.

The lack of effect of the mouth rinses when compared to the control may be related to the vehicle used to deliver the active ingredient and its mode of action. In this *in vitro* study, exposure to the mouth rinses did not occur under agitation, considering that under clinical conditions, they are usually swished around the mouth for a given period of time and expectorated afterwards. Although mouth rinses do come into direct contact with hypersensitive areas of the teeth, contrary to the way toothpastes are applied, mouth rinses are not rubbed on these areas with the toothbrush, therefore, penetration of the agents into the dentin tubules might be reduced.

One of the mouth rinses tested (Listerine Advanced Defense Sensitive) contains 1.4% potassium oxalate. Its mode of action relies on the formation of calcium oxalate crystals on the dentin surface and inside dentin tubules,[21] forming a physical barrier to the transmission of hydrodynamic stimuli.[14] Moreover, oxalates have been reported to be capable of providing the benefit of being acid-resistant.[22] A previous *in vitro* study[14] showed a significant reduction in dentin permeability after treatment with Listerine Sensitive, and this effect was confirmed by the cross-sectional SEM images that showed the calcium oxalate crystals filling the dentin tubules. The different outcome between the previous study cited and the present one could be attributed to the fact that, previously, treatment with the mouth rinse was performed at least 12 times, with only one acid challenge at the end of the entire treatment regimen, which was performed with lactic acid for 90 seconds. By contrast, in the present study, the test consisted of either a single application of the mouth rinses, or they were applied interposed with erosive and abrasive challenges; consequently, the mouth rinse was tested under a harsher condition. Worth noting is that under clinical conditions, potassium oxalate can have a neural desensitizing effect, which could not be evaluated with the model used in the present study.[11]

Another mouth rinse tested was Colgate Sensitive Pro-Relief, which relies on the Pro-Argin technology, and contains 0.8% arginine, 225 ppm F^-, PVM/MA copolymer, and pyrophosphate. This composition provided the formation of an arginine/copolymer complex that obliterated the dentin tubules, which may explain the reduction in permeability of dentin, as was previously observed *in vitro* and in clinical studies.[10,23-25] Arginine is a positively charged amino acid present in saliva, which binds to the dentin and has a negative charge. Under basic pH values, there is the deposition of arginine, calcium, phosphate, and

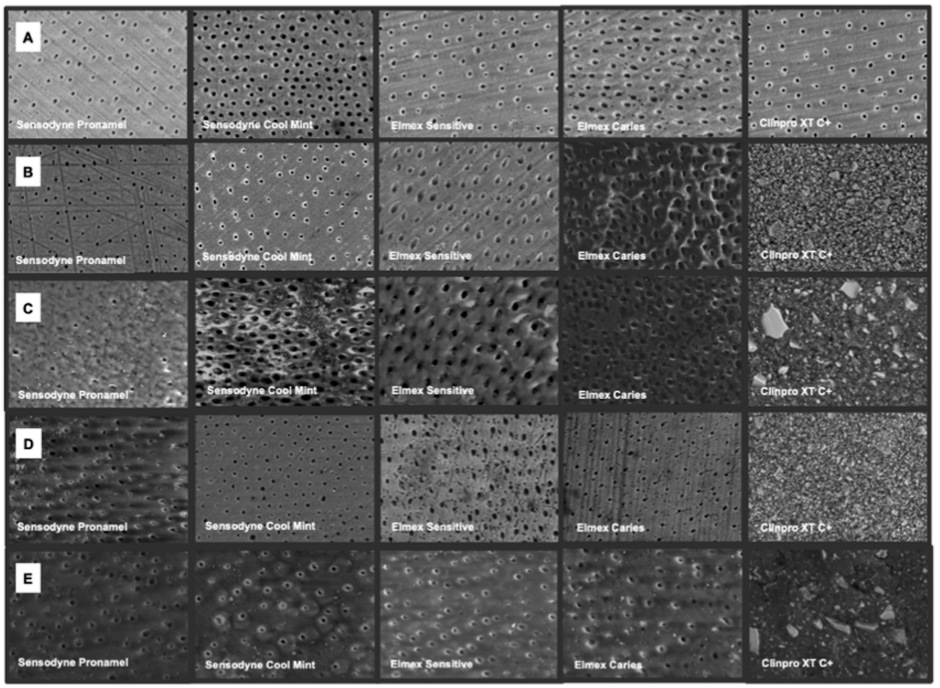

Figure 3. *(A) Dentin surface after 17% EDTA. (B) Dentin surface after treatment. (C) Dentin surface after erosion challenge showing a visibly worn treatment layer with dentin exposure. (D) Dentin surface after abrasion challenge showing a visibly worn treatment layer with dentin exposure. (E) Dentin surface after erosion/abrasion cycling showing a visibly worn treatment layer with dentin exposure. Control (C+) - Clinpro XT (C+), Sensodyne Pronamel, Sensodyne Cool Mint, Elmex Sensitive, Elmex Caries.*

carbonate on dentin and within dentin tubules.[26, 16] The copolymer PVM/MA has the ability to adhere to the dentin by van der Wall forces and by hydrogen bonding with surface proteins, acting by maintaining the active principles adhesively retained on the oral tissues.[27] Pyrophosphate stabilizes the complex in the solution and has the ability to deposit on the dentin surface.[10] Moreover, this mouth rinse was said to be able to form a coherent layer on the dentin specimens after multiple applications.[10] Corroborating this finding, in a clinical trial, it was observed that it could reduce dentin hypersensitivity to a larger extent than a fluoridated mouth rinse, after two weeks of use.[24] However, in our study, we could suggest that a single application prior to erosion or abrasion tests was not enough to produce significant tubule occlusion. During the cyclic conditions, the mouth rinse was applied more frequently, nevertheless, the number of ODTs found in this group was still no different from the negative control. It could be hypothesized that the erosive and abrasive episodes gradually removed the layer formed by the product and did not allow it to significantly occlude the dentin tubules.

Also based on the Pro-Argin technology, the mouth rinse Elmex Sensitive (ES) Professional (0.8% arginine, 250 ppm F^-, PVM/MA copolymer, and pyrophosphate) was not able to significantly seal the dentin tubules in the present study. By contrast, in a previous clinical study, the continuous use of the ES rinse was effective in reducing DH.[28] However, in the previous study cited, the rinse was combined with the use of the ES toothpaste, so the reduction in pain could not be exclusively attributed to the rinse itself. Furthermore, there was no negative control for comparison; therefore, a placebo effect cannot be disregarded.

The low concentration of fluoride in the rinses is another factor that may have influenced the results, as most of the rinses used in this study had an average of only 225 ppm F^-. With this concentration, low calcium fluoride-like deposits are expected, especially in neutral solutions.[29]

According to our results, only the positive control, Clinpro XT varnish (CXT; C^+), was able to promote significantly more tubular occlusion than the negative control (distilled water; C^-) after its application, and the challenges performed alone/separately, but not after cycling. Considering that there is no standard product to treat DH, we opted to use this product, based on the promising results that have been observed in previous studies.[30,31] CXT varnish is a resin-modified glass ionomer. The modified polyalkenoic acid copolymer present in its composition is responsible for a strong chemical union to the calcium of hydroxyapatite.[32] This chemical union might be the reason why the coating resisted throughout the erosive and abrasive challenges performed alone/separately and maintained the sealing of the dentin tubules. Another contributory factor could be the fluoride released from the particles of fluoro-alumino-silicate and calcium and phosphate present in calcium glycerophosphate.[33] However, the varnish did not withstand several erosive-abrasive episodes, as observed previously.[30] In agreement, some

open dentin tubules were visible in the micrographs of this group taken after cycling.

For this study, we opted to use environmental scanning electron microscopy analysis, because it does not require prior preparation of the specimens, allowing multiple readings of the same specimens at different time points, hence each specimen served as its own control. It is also one of the methods that has received most support for studying the *in vitro* effect of desensitizing agents.[34] By using the Image J program to count the number of tubules in the micrographs, it was possible to decrease the subjectivity of the analysis.[20] Efforts were made at all times to take the images in the same region of the specimens, allowing an accurate comparison between experimental time intervals.

One limitation of the present study was that only the number of open tubules was taken into account, disregarding the fact that a reduction in dentin tubule diameters could clinically result in reducing the pain of DH.[15] In addition, our study did not take into consideration the fact that under clinical conditions, not only is the rinse diluted with the patient's saliva, but it is also swished around the mouth, which could potentially allow increased formation of calcium fluoride-like deposits. Thus, care should be taken when extrapolating the findings of the present study to the clinical scenario. Nonetheless, this study highlights an important aspect about desensitizing mouth rinses, indicating that they should not be used as the only treatment to control DH. Further studies should evaluate their effects in association with other therapies, such as desensitizing toothpastes.

CONCLUSION

It could be concluded that none of the desensitizing mouth rinses tested was able to promote a significant occlusion of the dentin tubules both in the periods after treatment and after erosive and abrasive challenges.

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Regulatory Statement

This study was conduected in accordance with all the provisions of the human subejcts oversight committee guidelines and policies of the ethis committee on resarch involving human beings, CEP/FOUSP. The approval code issued for this study is 89906318.7.0000.0075.

Conflict of Interest

The authors have no financial interest in any of the companies or products mentioned in this article.

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