The effect of desensitizing dentifrices on dentin wear and tubule occlusion

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ABSTRACT: Purpose: To evaluate the effect of desensitizing dentifrices on dentin erosive wear, using a 5-day erosion-abrasion-remineralization cycling model. The effect of the dentifrices on dentin's tubule occlusion was also investigated. **Methods:** 30 samples of root dentin were randomly divided into three groups (n=10): (1) Colgate Total 12 Clean Mint (control, 1,450 ppm F); (2) Colgate Sensitive Pro-Relief (1,450 ppm F, Pro-Argin); and (3) Sensodyne Repair&Protect (1,450 ppm F, Novamin). Erosion was performed with a cola drink, for 5 minutes, 4×/day. Toothbrushing with the slurry dentifrices (1:2) was performed 2×/day, with electric toothbrushes, using standard pressure for 15 seconds. Surface loss (SL) was determined with optical profilometry at baseline and after the first, third and fifth days of cycling. Before treatment and in the end of the cycling, the amount of opened dentin tubules per area was evaluated in three randomly selected specimens from each group, by environmental scanning electron microscopy. The relative dentin abrasivity (RDA) of the dentifrices was also measured. Data were statistically analyzed ($\alpha = 0.05$). **Results:** All the dentifrices showed a progressive increase in SL over time. However, no significant differences in SL among the dentifrices were observed at any time studied. Sensodyne Repair&Protect significantly reduced the number of opened dentin tubules when compared to the other groups. Colgate Total 12 Clean Mint showed the highest RDA, followed by Sensodyne Repair&Protect and then by Colgate Sensitive Pro-Relief. The desensitizing dentifrices tested produced a similar rate of erosive dentin wear to the conventional dentifrice; however, only Sensodyne Repair&Protect was able to promote tubule occlusion. (*Am J Dent* 2015;28:297-302).

CLINICAL SIGNIFICANCE: The desensitizing dentifrices tested produced a similar rate of erosive dentin wear to the conventional dentifrice; however, only Sensodyne Repair&Protect was able to promote tubule occlusion.

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Introduction

Dental erosion can be defined as a superficial loss of the tooth surfaces caused by acids of non-bacterial origin.[1] When combined with the physical impacts of toothbrushing, it can result in excessive wear, which may negatively affect the long-term health of the dentition.[2,3] Initially, on enamel, the contact of the erosive acids with the tooth surface causes a superficial demineralization. This process is known as surface softening, because this demineralized layer presents reduced hardness and increased roughness,[4,5] and it is more vulnerable to physical impacts, such as toothbrushing abrasion;[6,7] and with progression, a bulk and permanent loss of the surface occurs.[4]

In dentin, the continuous mineral demineralization leaves the organic matrix exposed. This organic layer is known to be relatively resistant to the mechanical impact of toothbrushing, which has the ability to compress it, instead of removing it.[8,9] The effects of toothbrushing on eroded dentin may be less harmful than in the eroded enamel.[9] Clinically, it is unknown whether the organic matrix remains at the eroded dentin surfaces, but it is speculated that it is removed by the action of proteolytic enzymes present in the saliva.[10] When dentin becomes exposed, either by the loss of enamel that covers the crown, or at the radicular regions, a painful condition known as dentin hypersensitivity may also occur.[11]

Dentifrices contain anti-erosive agents, such as fluoride. Fluoridated toothpastes reduce erosive wear in enamel and dentin.[12-15] However, depending on the type of dentifrice used (mainly concerning its abrasiveness), toothbrushing with fluoridated toothpastes can fail to promote such effect.[16,17] Dentifrices have also been used to deliver desensitizing agents, aiming to reduce the pain associated with dentin hypersensitivity. These toothpastes can either contain desensitizing agents that have the ability to suppress nerve impulses by neurological interaction or by mechanical occlusion of the dentin tubules.[18]

Recently, a desensitizing dentifrice based on arginine (Pro-Argin[a]) was introduced. Arginine is an aminoacid frequently found in saliva, which in combination with calcium carbonate is thought to mimic the saliva's ability to seal dentin tubules.[19,20] A series of reports have shown that this dentifrice is also able to reduce enamel erosion and enhance remineralization of acid-softened enamel.[21,22] The combination of arginine and calcium carbonate penetrates the irregularities created by acid, forming a coating that prevents further acid attacks.[21] Another commercially available desensitizing dentifrice contains Novamin,[b] (or sodium calcium phosphosilicate (CSP), which is a bioactive glass-ceramic material that releases calcium and phosphate upon reaction.[23] It is believed that this material can physically occlude dentin tubules through the deposition of a hydroxyapatite-like mineral layer.[24,25] So far, the studies evaluating the effectiveness of this agent against enamel erosion have not been promising.[26,27]

Nevertheless, there is only little information about the effect of these two desensitizing substances against dentin erosion. This would be important, because dental erosion is one of the etiological factors of dentin hypersensitivity.[28] Thus, if the desensitizing dentifrice is not able to effectively control the progression of dentin wear from erosive and abrasive impacts, it may also have a short-term effect against dentin hypersensitivity.

In view of these aspects, the objectives of this study were: (1) To evaluate the effect of two desensitizing (containing arginine and CSP) and one conventional dentifrice on dentin erosion, using an erosion-abrasion-remineralization cycling model; and (2) To determine the degree of dentin tubule occlusion promoted by these dentifrices at the end of the cycling.

Materials and Methods

Experimental design - This study tested two experimental factors: dentifrices (at three levels – (1) Colgate Total 12[a] Clean Mint, as control group [1,450 ppm F, as sodium fluoride (NaF), without any active ingredient for the treatment of dentin hypersensitivity]; (2) Colgate Sensitive Pro-Relief[a] [1,450 ppm F, as sodium monofluorophosphate (MFP) and 8% arginine]; and (3) Sensodyne Repair&Protect[b] [1,426 ppm F as MFP and calcium sodium phosphosilicate (Novamin)] and experimental times at three levels: 1-day (T1), 3-day (T2), and 5-day (T3) cycling) in an erosion-remineralization-abrasion cycling model using dentin specimens (n= 10). The response variable was dentin surface loss (in µm), measured at baseline (T0) and after T1, T2, and T3 days of cycling. In order to test the ability of the dentifrices to occlude dentin tubules, three specimens were randomly selected from each group. Micrographs of the specimens were taken with environmental scanning electron microscopy after the treatment to open dentin tubules and simulate hypersensitive dentin, and at the end of the experiment. The micrographs were then quantitatively analyzed to determine the amount of open dentin tubules per area. Qualitative description of the micrographs was also performed. As an additional test, the relative dentin abrasivity (RDA) of the three dentifrices was evaluated, using the Hefferren abrasivity test recommended by the ADA, and ISO 11609 for determination of dentifrice abrasiveness in dentin.[29] All the analyses were performed in random sequence and in blind conditions.

Specimen preparation - Dentin slabs (4 mm × 4 mm × 1.5 mm) were cut from human third molars using a microtome (Isomet 1000[c]). The teeth were stored in 0.1% thymol solution, at 4°C, until the beginning of the experimental procedures. The slabs were embedded in acrylic resin (Varidur[c]) and the test surfaces were ground flat and polished (Ecomet 250[c] grinder-polisher), using aluminum oxide papers[c] (grits #600, #1,200 and #4,000), under water cooling; followed by diamond suspension (0.3 µm, Alfa Micropolish[c]) and felt paper. Between each paper and at the end of the polishing procedures, the specimens were sonicated[d] in distilled water for 3 minutes. After polishing, the specimens were evaluated under an optical microscope. Specimens with caries lesions or any other structural defect were discarded.

To simulate hypersensitive dentin, the specimens were immersed in 27% EDTA solution for 2 minutes. This procedure removed the smear layer and opened the dentin tubules.[30] Then, adhesive unplasticized polyvinyl chloride (UPVC) tapes were placed on the polished surface of each specimen, leaving an area of 4 mm × 1 mm exposed for subsequent testing.

The specimens were randomly divided into three experimental groups (n= 10), according to the dentifrice used for brushing: (1) Colgate Total 12 Clean Mint; (2) Colgate Sensitive Pro-Relief; and (3) Sensodyne Repair&Protect.

Erosive/abrasive challenges - The 5-day erosion-abrasion-remineralization model used was modified from Scaramucci et al.[15] The erosive challenge was performed with a cola drink (Coke,[e] pH of approximately 2.6). The specimens were immersed in the cola drink (5 ml/specimen), for 5 minutes, four times a day, without agitation and at room temperature; followed by a 60-minute immersion in artificial saliva (1.649 mmol/l $CaCl_2.H_2O$; 5.715 mmol/l KH_2PO_4; 8.627 mmol/l KCl; 2.950 mmol/l NaCl g/l; 92 mmol/l ITris Buffer; pH adjusted to 7 with HCl). After erosion and before saliva exposure, the specimens were rinsed with distilled water and gently dried with absorbent paper.

Toothbrushing 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 3000[f]), equipped with a pressure alert feature that signaled when the pressure had reached the value of 2.5 N. The head of the brush was positioned parallel to the surface of the specimens. Slurries of the dentifrices were prepared with distilled water (1:2 w/w). Total exposure time of the specimens to the dentifrice slurries, in each brushing episode, was 2 minutes. A single operator performed the toothbrushing procedures. To avoid contamination, each slurry had its own brush. Overnight, the specimens were stored in a humid environment, at 4°C.

Dentin surface loss evaluation - Surface loss was measured by an optical profilometer (Proscan 2100[g]), at four different experimental times: baseline (T0), after 1 day (T1), after 3 days (T2) and after 5 days (T3) of cycling. The baseline measurement was performed to select the specimens with curvature < 0.3 µm.[31] For the readings, the tapes were removed and an area 2 mm long (X) × 1 mm wide (Y) was scanned in the center of the specimens. The length covered both the treated area and reference surfaces. The step size was set at 0.01 mm and the number of steps at 200 in the (X) axis; and at 0.1 mm and 10, respectively, in the (Y) axis. The depth of the treated area was calculated based on the subtraction of the average height of the test area from the average height of the two reference surfaces by using the dedicated software (Proscan Application software v. 2.0.17[g]). For this measure, a 3-point height tool was applied.

Environmental scanning electron microscopy (ESEM) evaluation - In the beginning of the experiment, three specimens randomly selected from each group were analyzed by ESEM (Hitachi TM3000[h]), following the methodology described in Palazon et al[30] in order to verify, qualitatively and quantitatively, the number of opened dentin tubules. Briefly, representative micrographs were taken at ×2000, using 15 kVA, exactly in the center of the specimens. No sample preparation, such as coating with metal films, was required. These same specimens were re-evaluated after cycling.

Qualitative assessment - The micrographs had their surface characteristics evaluated and checked for the patency or occlusion of the dentin tubules.

Quantitative assessment - A computer program (Windows PowerPoint, version 7.0[i]) was used to standardize the micrographs for grading. The amount of opened dentin tubules were recorded by three different trained examiners and considered as the response variable. The mean number of

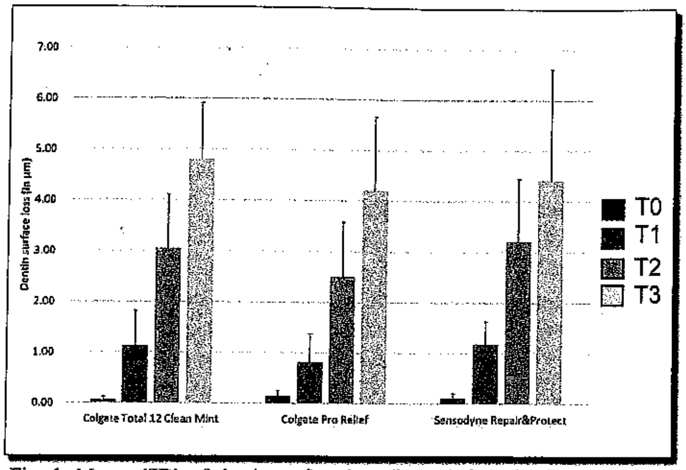

Fig. 1. Means (SD) of dentin surface loss (in µm) for all groups, in each experimental time. Different letters indicate significant difference among experimental times, for all the groups (P< 0.05).

opened dentin tubules was calculated for all groups using a representative micrograph for each group. For standardization purposes, open tubules, even with some occlusion causing a reduced diameter, were considered as open tubules.

Determination of the relative dentin abrasivity (RDA) of the dentifrices - An additional test evaluating the relative abrasion level (RDA) of the three dentifrices was performed. The RDA was determined by the radiotracer method recommended by the American Dental Association (ADA) and ISO 11609 for determination of dentifrice abrasiveness in dentin.[29] This test was performed at the Oral Health Research Institute, Indiana University School of Dentistry, according to the methodology previously described.[17]

Statistical analysis - Surface loss data were checked for normal distribution and homoscedasticity with Shapiro-Wilks and Brown-Forsythe tests, respectively. Since both statements were satisfied, two-way ANOVA and Tukey's tests were carried out for comparisons among groups. For the quantitative analysis of the ESEM micrographs, initially, the Friedman test was performed to check the consistency among investigators. Then, one-way ANOVA and Tukey's tests were carried out for comparison among groups. The RDA data were analyzed by one-way ANOVA and Student-Newman-Keuls tests. The significance level was set at 5%. The software SigmaPlot 13.0[j] was used for the calculations.

Results

Dentin surface loss - The dentin surface loss results, in each of the four experimental times (T0: baseline; T1: 1-day cycling; T2: 3-day cycling; and T3: 5-day cycling) are presented in Fig. 1. There was no significant interaction among the two studies experimental factors: dentifrices and time (P= 0.0904). Regarding the factor dentifrices, there were no significant differences among them, in all experimental times (P> 0.05). For the factor time, for all the groups, there was a significant increase in surface loss (P< 0.05) over the course of time, as follows T0<T1<T2<T3.

Quantitative analysis of the ESEM micrographs - In the quantitative analysis, the Friedman test showed a value of 0.098, which indicated reliability between the examiners. In the evaluation performed after the application of the 27% EDTA solution, all the specimens showed a similar pattern, with opened and widened dentin tubules. The mean (SD) of opened dentin tubules, for all the specimens used in this evaluation, was 76.93 (6.43). There was no significant difference in the number of opened dentin tubules of the specimens among the groups (P> 0.05). After cycling, Sensodyne Repair&Protect caused the greatest reduction on the number of open dentin tubules (mean±SD of open dentin tubules = 20±2.4), followed by Colgate Total 12 Clean Mint (mean±SD of open dentin tubules = 34± 1). Colgate Sensitive Pro-Relief was the dentifrice that exhibited the greatest number of opened dentin tubules per area (mean±SD of open dentin tubules = 56.89±2.45).

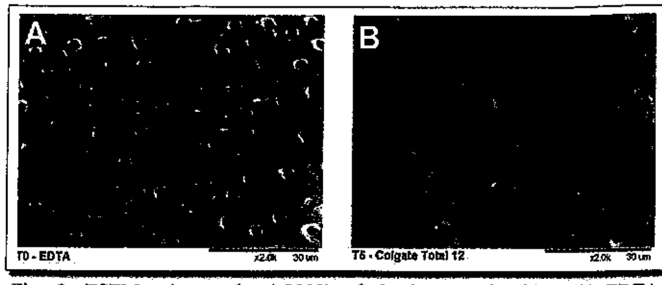

Fig. 2. ESEM micrographs (x2000) of dentin treated with 27% EDTA treatment, for 2 minutes, to simulate hypersensitive dentin (A) and after the 5-days cycling with Colgate Total 12 (B).

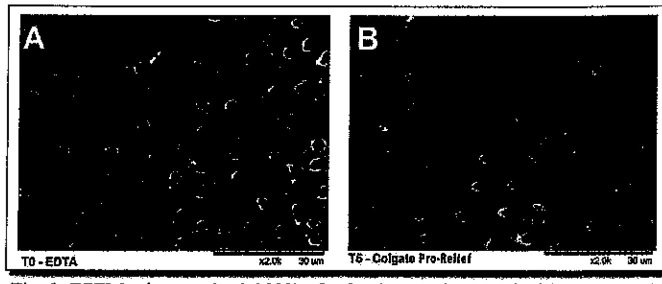

Fig. 3. ESEM micrographs (x2000) of a dentin sample treated with 27% EDTA treatment, for 2 minutes, to simulate hypersensitive dentin (A) and after the 5-days cycling with Colgate Pro-Relief, taken in end of the cycling (T3).

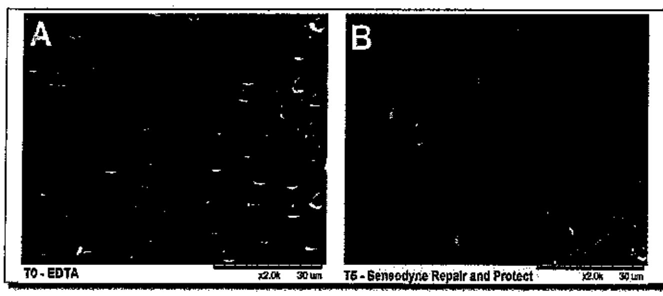

Fig. 4. ESEM micrographs (x2000) of dentin treated with 27% EDTA treatment, for 2 minutes, to simulate hypersensitive dentin (A) and after the 5-days cycling with Sensodyne Repair&Protect (B).

Qualitative analysis of the ESEM micrographs - The micrographs of all the groups in the two experimental times (baseline – T0/before treatment and after 5 days of cycling -T3) are shown in Figs. 1-3.

All micrographs A, from Figs. 2, 3 and 4, show the morphology of the samples after immersion in 27% EDTA for 2 minutes, to simulate hypersensitive dentin. A large number of dentin tubules per area can be seen. The EDTA solution was able to remove the smear layer, open and enlarge the diameter of the dentin tubules. All the specimens analyzed showed a similar pattern.

Figures 2B, 3B, and 4B show representative specimens from each group, after the final erosive-abrasive cycling model.

Figure 2B shows that, although Colgate Total 12 Clean Mint does not claim any desensitizing effect, occlusion or partial occlusion of the dentin tubules was observed. However, some opened dentin tubules were still visible. In Fig. 3B, Colgate Sensitive Pro-Relief was able to partially occlude the dentin tubules, reducing their diameter; however, the number of occluded tubules was higher than observed for Colgate Total 12 Clean Mint. In Fig. 4B, Sensodyne Repair&Protect promoted the most effective tubule occlusion, with almost no open tubule visible with a homogenous and amorphous layer over dentin.

Additional test - relative dentin abrasivity (RDA) - Colgate Total Clean Mint was the most abrasive toothpaste (RDA ± SD: 139.1 ± 4.51), followed by Sensodyne Repair&Protect (RDA ± SD: 102.6 ± 3.24). Colgate Sensitive Pro-Relief was the least abrasive dentifrice (RDA ± SD: 81.0 ± 3.55).

Discussion

In this study, all dentifrices tested showed a similar degree of dentin loss after the erosive-abrasive challenges, despite having different levels of RDA. According to the RDA test, Colgate Total Clean Mint was the most abrasive toothpaste, as it showed a RDA value 27% higher than Sensodyne Repair& Protect and 42% higher than Colgate Sensitive Pro-Relief. Some components of the formulation of Colgate Total Clean Mint and Sensodyne Repair&Protect may have contributed to prevent untoward damage caused by their higher abrasive system on eroded dentin.

According to Addy & Shellis,[32] a major factor in dentin wear is the abrasivity of the dentifrices, measured by RDA, which is the abrasivity of the dentifrice in relation to a standard paste, with the RDA set at 100. These authors also stated that the ISO (International Standards Organization) requires that the RDA of marked toothpastes should be less than 250. Although the RDA test is not meant to measure surface loss of eroded dentin, some studies have found a positive association between these variables.[33,34] Nevertheless, it is often difficult to relate the RDA values with dentin wear when the toothpaste contains agents with anti-erosive effect.[35] It can be hypothesized that the type of fluoride present in the dentifrices had some influence on the surface loss results of the present study.[17] Colgate Total 12 Clean Mint has sodium fluoride (NaF) in its composition, while the other toothpastes have sodium monofluorophosphate (MFP). Even though their fluoride concentrations are similar, it was observed that for in vitro conditions, NaF dentifrices presented better fluoride release than the dentifrices containing MFP.[36] Thus, the greater amount of fluoride available for Colgate Total 12 Clean Mint toothpaste may have compensated for its higher abrasivity. For Sensodyne Repair&Protect, it can be speculated that the deposition of the hydroxyapatite-like mineral layer caused by CSP[37] played a role in reducing the amount of eroded dentin removed by toothbrushing. Although there are some studies[26,27,38,39] evaluating the effect of CSP-containing dentifrices on enamel erosion, less information is available about the impact of these dentifrices on dentin. Parkinson & Willson[40] observed than a CSP-containing fluoridated dentifrice caused significantly more dentin surface rehardening that a dentifrice with similar F concentration. This finding supports our theory that CSP may have acted on the reduction of the softened dentin removed in each brushing episode.

Concerning Colgate Sensitive Pro-Relief, similar to Sensodyne Repair&Protect, there are some studies testing its anti-erosive effect on enamel,[22,41] but only a few on dentin. Although the mechanism in which arginine protects the enamel still needs to be further elucidated, it was suggested[21] that, when arginine combines with calcium, it would form a coating over the enamel surface reducing demineralization. However, considering that in this study the arginine-containing toothpaste presented the lowest RDA value, yet it showed similar erosive dentin loss to the other groups, one may suppose that, for dentin, this coating might be not enough to prevent demineralization. This hypothesis was formulated based on the fact that dentin has less mineral content than enamel, in addition to a more irregular surface, which may have interfered with the interaction of the surface protective agents.[42]

This study also showed that Sensodyne Repair&Protect was able to significantly reduce the number of open dentin tubules, while Colgate Sensitive Pro-Relief did not. A similar outcome for the CSP-dentifrice was observed.[40,43] In these two in vitro studies, the CSP-dentifrice was able to occlude and mineralize the hypersensitive-simulating dentin. In the present study, ESEM micrographs of the specimens treated with CSP showed a homogenous layer on the dentin surface, occluding the dentin's tubules. Besides tubule occlusion, the potential of CSP-dentifrices to reduce the pain in dentin hypersensitivity has also been demonstrated.[44,45] The Colgate Sensitive Pro-Relief was not able to reduce the number of open dentin tubules, but reduced their diameter, as can be seen on the ESEM micrographs. This reduced diameter might imply some desensitizing effect in clinical conditions. However, as observed in the in situ study of Olley et al,[46] although the arginine dentifrice presented similar tubule occlusion to a strontium acetate dentifrice, it was more susceptible to repeated acid challenges. Thus, we may assume that, in this present study, the calcium-arginine deposits created by Colgate Sensitive Pro-Relief was less resistant to the washing procedures performed after brushing and to the acid challenges than the ones created by Sensodyne Repair&Protect. Interestingly, as can also be observed in the ESEM micrographs, the toothpaste Colgate Total 12 Clean Mint was able to promote some tubule occlusion. This could be related to the presence of abrasive granules in its composition, which may have entered the dentin tubules forming plugs.

The present in vitro study used an erosion-abrasion-remineralization cycling model, which intended to simulate the clinical situation of individuals suffering from dentin hypersensitivity, who brush their teeth twice a day and consume acidic beverages with high frequency. This model was similar to that of a previous investigation,[15] where a dose-response effect was found between the control dentifrice and a placebo dentifrice (containing 1,100 ppm F and 0 ppm F, respectively). This previous result supports the fact that the model is sensitive and selective in differentiating treatments; therefore, was chosen to be used in this investigation. To simulate hypersensitive dentin, the method proposed by Palazon et al[30] was used. In this study, the authors tested three methods available in the literature to open dentin tubules and simulate hypersensitive dentin. It was concluded that the solution that promoted the best simulation of hypersensitive dentin, with efficient removal of the smear layer and opening of dentin tubules was the 27% EDTA, applied for 2 minutes; and thus was the protocol chosen in the present investigation. The electric toothbrush used was equipped with a pressure alert that signaled when the pressure had reached the value of 2.5 N, which is within the range of force recommended for erosion-abrasion studies.[47]

One limitation of this study would be the preparation of the slurries, which were mixed with distilled water instead of artificial saliva. Since the artificial saliva had calcium in its composition, it is possible that in clinical conditions, additional sources of this ion would be beneficial for a better action of the desensitizing agents. However, similar to previous investigations,[41,48] in this study we opted to prepare the slurries with distilled water instead of saliva to avoid the agents reacting during mixing, before reaching the dentin specimens. In this sense, caution should be taken when extrapolating the findings of this study to the clinical scenario.

Within the limitations of this in vitro study, it was concluded that the desensitizing dentifrices tested presented similar erosive dentin wear rate to the conventional fluoridated dentifrice with no desensitizing agent. The desensitizing dentifrice containing calcium sodium phosphosilicate (Sensodyne Repair &Protect) was the only dentifrice capable of promoting tubule occlusion at the end of the erosive-abrasive challenges.

a. Colgate, New York, NY, USA.
b. GSK, Brentwood, Middlesex, UK.
c. Buehler Ltd, Lake Buff, IL, USA.
d. Kondortech, São Carlos, SP, Brazil.
e. The Coca-Cola Company, Porto Real, RJ, Brazil.
f. Oral B, Schwalbacham Taunus, Germany.
g. Scantron, Tauton, England, UK.
h. Hitachi, Tokyo, Japan.
i. Microsoft, Redmond, WA, USA.
j. Systat Software Inc., San Jose, CA, USA.

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Articles Accepted for Publication

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