Effect of different toothpastes on permeability and roughness of eroded dentin

Fernanda SS Ramos¹, Alberto CB Delbem¹, Paulo H dos Santos², Mariana D Moda¹, André LF Briso¹, Ticiane C Fagundes¹

- 1. Universidade Estadual Paulista (UNESP), Faculdade de Odontologia, Departamento de Odontologia Preventiva e Restauradora, Araçatuba, SP, Brasil.
- 2. Universidade Estadual Paulista (UNESP), Faculdade de Odontologia, Departamento de Materiais dentários e Prótese, Aracatuba, SP, Brasil.

ABSTRACT

Dentin hypersensitivity (DH) is characterized by rapid, acute pain arising from exposed dental tubules. Aim: the aim of this study was to evaluate the roughness, tubule occlusion, and permeability of eroded dentin brushed with different toothpastes. Materials and Method: ninety bovine teeth were cut into blocks. Thirty hemifaces were protected with varnish and the other sixty were submitted to permeability tests. Specimens were divided into groups according to the dentifrices: without fluoride (WF), sodium fluoride (NaF), and stannous fluoride (SnF₂). The blocks were subjected to a 5-day erosive-abrasive protocol. Surface roughness and dentinal tubule occlusion (n=10) were assessed for both control and test hemifaces of the same sample along with permeability analysis (n=20). Two-way RM ANOVA and Tukey's post-hoc test were performed ($p \le 0.05$). **Results:** NaF and SnF, presented higher roughness than WF. The number of open tubules was higher in WF. Permeability was higher in SnF., but there was no significant difference between WF and NaF. Conclusions: both fluoride toothpastes occluded dentinal tubules and increased roughness. NaF toothpaste promoted greater decrease in dentin permeability.

Keywords: dentifrices - dentin desensitizing agents - dentin permeability - tooth abrasion - tooth erosion

Efeito de diferentes cremes dentais na permeabilidade e rugosidade da dentina erodida

RESUMO

A hipersensibilidade dentinária (HD) é caracterizada por dor rápida e aguda decorrente de túbulos dentais expostos. Objetivo: este estudo teve como objetivo avaliar a rugosidade, oclusão tubular e permeabilidade da dentina erodida escovada com diferentes dentifrícios. Materiais e Método: noventa dentes bovinos foram seccionados em blocos. Trinta hemifaces foram protegidas com verniz e outras sessenta foram submetidas à permeabilidade. Os espécimes foram divididos em grupos de acordo com os dentifrícios: sem flúor (SF), fluoreto de sódio (NaF) e fluoreto estanoso (SnF.). Em seguida, os blocos foram submetidos a um protocolo erosivo-abrasivo de 5 dias. A rugosidade da superfície e a oclusão do túbulo dentinário (n = 10) foram avaliadas para ambas as hemifaces de controle e teste da mesma amostra, também realizou-se a análise de permeabilidade (n = 20). Two-way RM ANOVA e pós-teste de Tukey foram realizados (p≤0,05). **Resultados:** NaF e SnF, apresentaram rugosidade superior ao SF. O número de túbulos abertos foi maior em SF. Não encontramos diferenças significativas entre SF e NaF em relação à permeabilidade; entretanto, SnF, apresentou maior permeabilidade. Conclusão: ambos os dentifrícios fluoretados foram capazes de ocluir os túbulos dentinários e aumentar a rugosidade. No entanto, o dentifrício NaF promoveu uma maior diminuição da permeabilidade dentinária.

Palavras-chave: dentifrícios - agentes dessensibilizantes da dentina - permeabilidade da dentina abrasão dentária - erosão dentária

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Corresponding Author:

Ticiane Cestari Fagundes ticiane.fagundes@unesp.br

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INTRODUCTION

beverage intake³.

Dentin hypersensitivity (DH) is characterized by rapid, acute pain arising from exposed dental tubules in response to thermal, tactile, chemical, and osmotic stimuli¹. According to the hydrodynamic theory, physical stimuli promote the movement of fluids within dentin tubules, contracting and expanding odontoblastic processes and stimulating nerve fibers present in the dentin-pulp interface². DH affects patients' oral health and quality of life, and is one of their main complaints¹. The etiology of DH is multifactorial and often associated with non-carious cervical lesions (NCCL)1,3, whose prevalence is about 11.5%, regardless of the etiology. Molars and premolars are the teeth most frequently affected by NCCL, with incidence and severity increasing with patient age⁴. The depth

and morphology of lesions contribute to high DH

levels, and are affected by gastric disease and acidic

Dental erosion is one of main factors involved in DH. It results from the loss of surface tooth tissue due to the action of acids from intrinsic or extrinsic sources^{5,6}. Acids from intrinsic sources affect the palatal surface of anterior teeth and the occlusal surface of posterior teeth, whereas those from extrinsic sources affect the vestibular and cervical surfaces of anterior teeth⁶. The action of acids and proteases changes to dissolve minerals, glycoproteins and other components responsible for occluding dentinal tubules⁷. Reduced salivary flow, either due to medication use or xerostomia, makes it difficult to restore the oral pH and, consequently, contributes to the erosive process³.

Desensitizing and anti-erosive toothpastes are indicated for treating DH^{8,9}. However, their beneficial effects are limited, with results ranging from no action to almost complete desensitization and erosion inhibition, depending on the type. High-quality studies are therefore needed².

Different components have been added to toothpastes with the aim of increasing the resistance of the dental substrate to erosive processes and/or dentin tubule obliteration^{2,10,11}. Dentifrices containing polyvalent metal ions, such as stannous ions, have effectively reduced the erosion process by depositing ions on eroded tissue, thereby preventing contact with acids^{2,10,12,13,14}. Conversely, abrasive compounds are also often added to dentifrices to improve their cleaning potential¹⁵, which may influence the

exposure of dentin tubules after erosion-abrasion cycles, increasing permeability and roughness, which have been associated with the development of DH^{16,14}. Although some studies have evaluated dentifrice action on eroded dentin^{17,18}, there are few studies that have simultaneously evaluated roughness, permeability, and tubular obliteration with the use of sodium fluoride- and stannous fluoride-based dentifrices. The aim of this study was therefore to evaluate eroded dentin properties after erosive-abrasive challenge using three different commercial dentifrices. The hypothesis was that there are no differences between dentifrices in protecting against erosive-abrasive cycles regarding roughness, tubular obliteration, and dentin permeability.

MATERIALS AND METHODS

The project was approved by the Ethics Committee on the Use of Animals of Araçatuba School of Dentistry (#00414-2018).

Experimental design

The following factors were assessed: (1) two kinds of bovine dentin (sound and eroded); and (2) three dentifrices – fluoride free (without fluoride - WF-negative control – Curaprox Enzycal Zero rybol AG Neuhausen as Rheinfall. Switzerland); with sodium fluoride (NaF - positive control, 1450 ppm NaF Colgate Total 12, Colgate Palmolive, São Bernardo do Campo, SP, Brazil); and with stannous fluoride (SnF₂; 1100 ppm F as SnF₂ Crest Pro-Health, P&G, Cincinnati, Ohio, USA). Table 1 shows the composition and characteristics of each dentifrice. Response variables were surface roughness, dentinal tubule occlusion assessed using scanning electron microscopy (SEM), and dentin permeability. Figures 1 and 2 shows flowcharts of the experiments.

Preparation of dentin specimens

Ninety bovine incisors from 23 animals aged 24 to 30 months were extracted, cleaned, and stored in distilled water until the beginning of the experiment, for a maximum time of 60 days. Any teeth with clinical evidence of caries, root resorption, cracks, or fractures were excluded from the study. Dental crowns were separated from the roots using an IsoMet 1000 precision cutting saw (Buheles, IL, USA) with diamond disc (4" × 0.12" × ½", Buehler, Illinois, USA) under water cooling.

Table 1. Dentifrices used in the study and their composition according to manufacturers.				
Material	Tipe	Code	Composition	Manufacture
Curaprox Enzycal Zero (RDA-60) † Batch:442MHDEXP1121	Fluoride-free Toothpaste	WF	Water, Sorbitol, Hydrated Silica, Glycerin, Steareth-20, Titanium Dioxide (CI 77891), Aroma, Sodium Phosphate, Carrageenan, Sodium Chloride, Citric Acid, Sodium Benzoate, Potassium Thiocyanate, Glucose Oxidase, Amyloglucosidase, Lactoperoxidase	Trybol AG, Neuhausen AM Rheinfall, Swiss.
Colgate Total 12 (RDA-70/80) † Batch:6184BR121R	Sodium Fluoride Toothpaste	NaF	Sodium Fluoride (1450 ppm as NaF) Water, Triclosan, Sorbitol, Silica, Sodium Lauryl Sulfate, PMV / MA Copolymer, Sodium Hydroxide, Saccharin Sodium, Titanium Dioxide	Colgate- Palmolive, São Bernardo do Campo, SP, Brazil.
Crest Pro-Health (RDA-155) † Batch:6039GF	Stannous Fluoride Toothpaste	SnF2	Stannous fluoride (1100 ppm F as SnF2) Glycerin, Hydrated Silica, Sodium Hexametaphosphate, Propylene Glycol, PEG 6, Water, Zinc Lactate, Trisodium Phosphate, Sodium Lauryl Sulfate, Sodium Lauryl Sulfate, Carrageenan, Sodium Saccharin, Xanthan Gum, Blue 1	P&G, Cincinnati, Ohio, USA.
†RDA values according to manufacturers				

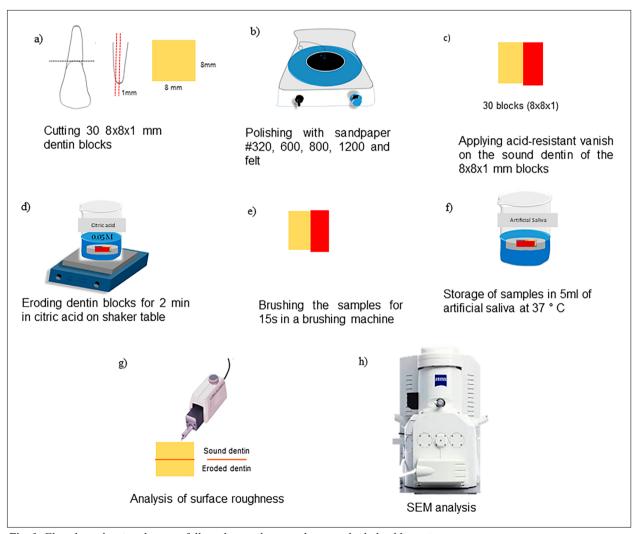


Fig. 1: Flowchart showing the steps followed to analyze roughness and tubule obliteration

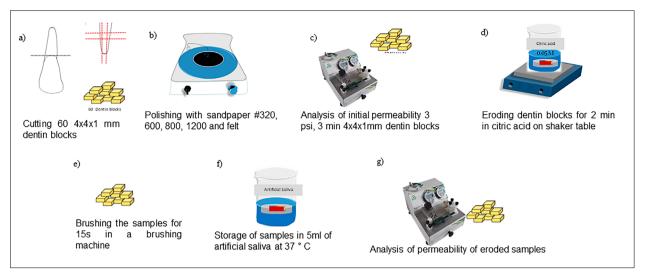


Fig. 2: Flowchart showing the steps followed to analyze dentin permeability

Sixty roots were cut with diamond disc $(4" \times 0.12" \times \frac{1}{2}"$, Buehler, Illinois, USA) under water cooling to obtain dentin blocks $(4 \times 4 \times 1 \text{ mm})$, and abraded with sandpaper discs of decreasing grit (# 320, # 600, # 800, and #1200) until they were 1 mm thick, and prepared to undergo initial dentin permeability analysis.

The other thirty roots were sectioned transversely into blocks 1 mm thick (8 \times 8 mm), which were polished with silicon carbide sandpaper discs of decreasing grit (# 320, # 600, # 800, and # 1200) using a polisher (Aropol E, Arotec, Cotia, SP, Brazil). The final polishing was performed using felt disc and diamond polishing paste (1 μ m, Arotec APL4), and surfaces were coated with an acid-resistant varnish (Colorama, São Paulo, SP, Brazil), to serve as a reference area (sound dentin).

Initial analysis of dentin permeability

To standardize dentin blocks, the 60 specimens ($4 \times 4 \times 1$ mm) underwent initial dentin permeability analysis. Specimens were immersed in a 37% phosphoric acid solution (1 ml/block) for 30 seconds, washed twice for 1 minute in deionized water, and dried with absorbent paper to remove the smear layer. Cellular and extracellular contents were removed from inside dentinal tubules by immersing the blocks in a 10% NaOH solution (3 ml/block) for 6 hours, followed by deionized water (3 ml/block) for a further 6 hours 19,20 .

Each specimen was coupled to a fluid infiltration system (Dentin Permeability, Odeme, Luzerna, SC, Brazil) with 1.9 psi initial water pressure. An

air bubble was inserted in the capillary tube, and the pressure was increased to 3 psi, stabilizing the air bubble for 30 seconds. After stabilization, the flow through the block was recorded for 3 minutes by recording the bubble displacement within the capillary tube using a 1 mm-resolution digital caliper. The displacement, expressed in mm, provides the flow rate (Q) – volume of deionized water passing through dentinal tubules -, determined by the following formula: $Q = (Vs \times D)/(L \times T)$, where Vs is the standard volume of the capillary tube in µl, D the bubble displacement in mm, L the capillary length in mm, and T the time in minutes. The dentin permeability (Lp), expressed in $\mu l.cm^2/min.cm H_2O$, is calculated based on the filtration rate (Q), using the following formula: $Lp = Q/(PH \times Asup)$, where PH is the hydrostatic pressure and Asup the exposed dentin surface area^{16,20}. Specimens with initial dentin permeability between 1.5 and 2.5 µl.cm²/min.cm H₂O were selected.

Erosion – abrasion process

For the erosion process, thirty blocks ($8 \times 8 \times 1$ mm) were coated with an acid-resistant varnish (Colorama, São Paulo, SP, Brazil), serving as reference area (sound dentin) prior to the challenge. The other sixty blocks ($4 \times 4 \times 1$ mm) underwent the challenge after initial permeability had been determined. The erosion process consisted of treating the specimens using an orbital shaker table (Tecnal TE -420, Piracicaba, SP, Brazil), while immersed in 0.05 M citric acid solution (Merk Biochemistry, Damstadt, Germany) (pH 3.2 - 2 ml/specimen) for 2 minutes, 4 times per

day, with 1-hour intervals between each cycle, for 5 days²¹. After the first and last erosive cycles of the day, specimens were brushed with the evaluated toothpastes for 15 seconds (1:3 – toothpaste/distilled water – slurry) using an automatic brushing machine, 45 strokes, 2 N force (MSET, Elquip, São Carlos, SP, Brazil) perpendicular to the specimen surface (extrasoft Condor, round bristles, São Bento do Sul, SC, Brazil) and immersed in slurry for 2 minutes at room temperature²¹. After each cycle, specimens were rinsed in distilled water for 30 seconds and stored in a remineralizing solution (artificial saliva: 1.5 mmol.l⁻¹ Ca(NO₂), 4H₂O; 0.9 mml.l⁻¹ NaH₂PO₄.2H₂O; 150 mmol.l⁻¹ KCl, 0.1 mol.l⁻¹ Tris buffer; pH 7.0) at 37 °C. Specimens were kept in artificial saliva at 37 °C at the end of each experimental day and stored in 100% humidity, with distilled water at 37 °C at the end of the erosive-abrasive protocol until they were analyzed.

Analysis of surface roughness

Surface roughness was analyzed on the $8\times8\times1$ blocks. Each specimen presented half sound and half eroded dentin - both analyzed after cycling (Surftest SJ 401 Rugosimeter - Mitutoyo, Mitutoyo American Corporation, Aurora, Illinois, USA) according to the Ra roughness pattern, which is the arithmetic mean between peaks and valleys. The cut-off used to maximize surface undulation filtering was 0.25

mm. Three equidistant readings were made on each surface and averaged²².

Analysis of dentinal tubule obliteration

Three specimens $(8 \times 8 \times 1 \text{ mm})$ per group were fixed in gold-covered metal stubs (SCD 050, Balzers) and subjected to scanning electron microscopy (EVO HD LS-15, Carl Zeiss do Brasil Ltda, SP, Brazil), at 15 kV and ×2000 magnification. The number of obliterated tubules was calculated for both sound and eroded surfaces in the same specimen, starting at the center of the sample and following northwest and southeast directions. A representative image of the specimen was used for quantitative analysis of dentinal tubules using the ImageJ software (National Institutes of Health, Betseda, MD, USA). Total number of tubules was determined by counting all the tubules present in the image (Fig. 3A), and the percentage of unblocked tubules was calculated23-24.

Final analysis of dentin permeability

The final analysis of dentin permeability was performed after the erosive-abrasive cycles, as described above.

Statistical analysis

Surface roughness, tubular obliteration, and dentin permeability showed normal distribution

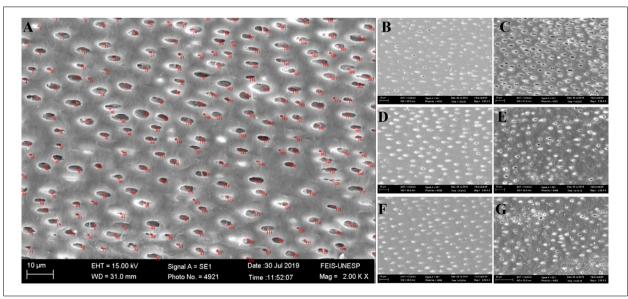


Fig. 3: Example of Image J software for counting open tubules. Dentin surface on the sound and eroded dentin.A: Tubule count B: WF sound dentin group; C: WF eroded dentin group; D: NaF sound dentin group; E: NaF eroded dentin group; F: SnF₂ sound dentin group; G: SnF, eroded dentin group.

(Shapiro-Wilk test) and homogeneity (Cochran test). Data on bovine dentin (sound and eroded) and dentifrices were submitted to two-way repeated measures ANOVA, followed by Tukey's post-hoc test. Statistical analysis was performed using the SigmaPlot 12.0 software (System Software, San Jose, CA, USA), with 5% significance level.

RESULTS

Figure 4 presents the results of the surface roughness analysis (Ra). The WF toothpaste showed the lowest roughness after the erosive-abrasive cycles (p=0.001), with values significantly different from those recorded for the fluoride toothpastes, which were statistically similar to each other (p=0.735). After the erosive-abrasive cycles, all the tested toothpastes promoted a significant increase in dentin surface roughness (p<0.001).

Figures 3 and 5 show the results of the analysis of dentin tubule obliteration using scanning electron microscopy (SEM). No difference was found between toothpastes for sound dentin ($p \ge 0.05$); however, the WF group had a significantly greater number of unblocked dentin tubules (84%) after the erosive-abrasive cycles compared to the NaF (49.2%) and SnF₂ (61.4%) groups (p<0.001), which were statistically similar (p=0.201). All groups presented a significantly increased number of unblocked tubules after the erosion-abrasion cycles $(p \le 0.05)$. Figure 3 shows an alteration in specimen surface induced by the action of citric acid. In the group comparison, WF presented the greatest number of open dentinal tubules and SnF, presented some granules deposited on the surface.

Figure 6 shows the results of dentin permeability.

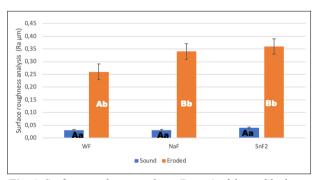


Fig. 4: Surface roughness analysis (Ra µm) of dentin blocks according to the treatment group and the specimen area (sound and eroded). Different letters (uppercase between groups and lowercase intragroup) indicate statistically significant differences (p < 0.05). WF: toothpaste without fluoride. NaF: toothpaste with sodium fluoride. SnF2: toothpaste with stannous fluoride.

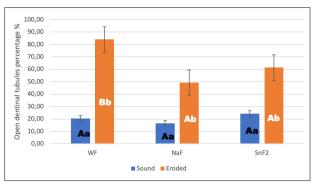


Fig. 5: Dentinal tubules occlusion – SEM evaluation: values of open dentinal tubules, as a percentage of sound and eroded dentin. Different letters (uppercase between groups and lowercase intragroup) indicate statistically significant differences (p <0.05). WF: toothpaste without fluoride. NaF: toothpaste with sodium fluoride. SnF2: toothpaste with stannous fluoride.

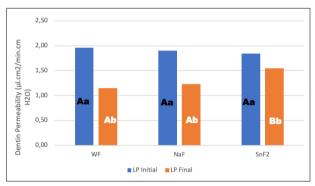


Fig. 6: Dentin permeability according to dentifrice (μ l.cm²/min. cm H2O). Different letters (uppercase between groups and lowercase intragroup) indicate statistically significant differences (p < 0.05). Lp = dentin permeability. WF: toothpaste without fluoride. NaF: toothpaste with sodium fluoride. SnF2: toothpaste with stannous fluoride.

No statistical difference was found between WF and NaF toothpastes (p=0.912, 45% and 40% reduction, respectively), but SnF₂ showed a 14% reduction in dentin permeability after erosive-abrasive cycles when compared to the other dentifrices (p<0.001). All three dentifrices decreased dentin permeability after the erosive-abrasive cycles (p<0.001).

DISCUSSION

Since one of the main etiologic factors of dentin hypersensitivity (DH) is erosion⁵, various studies have analyzed several types of dentifrices with desensitizing and anti-erosive components to evaluate their action on the dentin surface^{2,8,9,17,18,25-27}. In the current study, in order to ascertain whether dentifrice fluoride content affected the results, a toothpaste without fluoride (WF) was selected as a negative control. The toothpaste containing sodium

fluoride (NaF) was selected as a positive control based on studies that report its protective capacity on the eroded tooth surface^{10,18,25,28}. The toothpaste containing stannous fluoride (SnF₂) was selected because studies indicate that its components may cause superficial obliteration of dentinal tubules^{2,18,27,29}, thus addressing the need to assess tubular obliteration and dentin permeability^{11,16,24}. Surface roughness was analyzed to evaluate the influence of toothpaste abrasiveness on the surface of the eroded and abraded substrates.

Dentifrices contain abrasive particles, mostly silica derivatives, as polishing agents^{15,30}. These materials are added to toothpastes to eliminate bacterial biofilms and reduce other debris accumulation on tooth surfaces, but they may increase dentin wear by increasing toothpaste slurry abrasivity (relative dentin abrasivity or RDA)^{30,31}. The WF toothpaste caused significantly lower roughness than others, probably due to its lower RDA value³². The NaF and SnF, dentifrices promoted similar roughness and wear on eroded dentin, which may be explained by the amount of silica and the chemical influence of other components present in the NaF-based toothpaste^{10,30}. After simulating toothbrushing abrasion equivalent to two years, Aguiar et al.32 found that dentifrices with RDA values similar to those of SnF₂-based toothpastes cause roughness similar to that caused by the NaF dentifrice, in agreement with our results³².

Surface roughness was higher in eroded dentin than in sound dentin for all tested toothpastes. Tooth surfaces with values above 0.2 and 0.3 µm (after cycling) increased bacterial adhesion to dentin surface³³. The literature reports that toothpastes may increase surface roughness 8-fold^{32,33}. This finding corroborates our results, where roughness increased by 8 to 10 times as specimens underwent both abrasive and erosive action.

In addition to silica, toothpastes with desensitizing or anti-erosive action contain particles capable of obliterating dentinal tubules, which can be achieved either by an occluding layer on top of the dentin or by depositing material inside the tubules²⁵. Scanning electron microscopy (SEM) showed a high percentage of open dentinal tubules after the erosive-abrasive challenge, especially in the WF group, mainly because toothpaste WF does not contain functional agents capable of inducing tubular occlusion or intratubular mineralization²⁵. Studies on SnF₂-based toothpastes

have reached controversial results, since some found it to be superior to conventional toothpastes^{12,13,14,29}, whereas others reported similar results between them^{25,28}, as does our study. Some studies reported the presence of SnF₂ particles on the substrate surface, obliterating the top of dentinal tubules^{18,25}, but found no particles within tubules when they were analyzed in cross-sections²⁵. According to a study by João-Souza et al.³⁴, stannous has no protective effect against dental erosion and abrasion but helps reduce open tubular areas.

The current study also verified a significant increase in the number of open tubules for both sound and eroded dentin in all groups. This is due to exposure to citric acid. Frequent consumption of citric acid leads to tooth structure loss and smear layer removal¹⁶. Using an experimental dentifrice with sodium trimetaphosphate associated with fluoride, Favretto et al.¹⁹ found a greater number and larger internal area of open tubules by SEM analysis in placebo groups subjected to erosive challenges, as occurred in the WF group in our study¹⁹.

Although SnF₂ caused dentin obliteration similar to that of NaF, it promoted a significantly higher permeability of eroded dentin compared to the other dentifrices after cycling. This may be due to tissue wear resulting from toothpaste abrasiveness, leading to a loss of substrate thickness and possibly interfering with its desensitizing effect²⁵. According to Arnold et al.²⁵, a dentin thickness of approximately 61.98 µm may be lost after an erosive cycle performed with highly abrasive stannous-based dentifrices, which may contribute to dentin sub-surface analysis. Another study found that a SnF, dentifrice was unable to reduce dentin permeability, in contrast to the NaF dentifrice and two other toothpastes with desensitizing action (one containing calcium silicate and sodium phosphate, and another with potassium nitrate)^{17,25}. Dentinal obliteration can be dissolved by acids²⁵, and the high concentration of abrasive components in SnF₂ dentifrices may favor stannous ion bonding with silica, reducing its antierosive action¹⁵. A meta-analysis found that SnF₂based toothpaste achieved better outcomes than other compounds within a period of up to 2 weeks. However, it lost effectiveness at the 4 and 8-week follow-up, presenting a rapid desensitizing effect⁸. Another systematic review found that toothpastes containing SnF, reduced DH effects after 8 to 15 days of brushing²⁷. The use of artificial saliva and

the absence of dental biofilm and salivary film may reduce fluoride retention on surfaces in 5-day *in vitro* erosive protocols³⁵, which are supposedly more aggressive than an *in vivo* cycling protocol for the same period.

Although fluoride has beneficial effects in treating caries lesions, its actions on the erosive process are not as noticeable²⁶. In the current study, the NaF-based dentifrice contains 350 ppm more fluoride (F) than SnF₂. Despite the reduced action of fluoride in the erosive process, this difference may have influenced the lower permeability found in the SnF₂ group in relation to NaF. These results contradict those reported in another study that evaluated NaF and SnF₂ dentifrices, which found decreased dentin permeability for both¹⁸.

The group treated with WF toothpaste showed a greater number of open tubules, but lower permeability in eroded dentin, which may be explained by the deposition of calcium phosphate from saliva and a smear layer on the dentin surface^{20,36}. From the results of a meta-analysis that evaluated 30 randomized clinical trials, it was concluded that conventional fluoride toothpastes showed no significant difference in desensitizing

CONCLUSIONS

Our findings show that the two fluoride-based dentifrices are equally able to occlude dentinal tubules and increase the roughness of the eroded dentin, and that the sodium fluoride toothpaste promoted a greater decrease in dentin permeability.

effect when compared to placebo⁸. According to João-Souza et al.³⁴, the abrasion resulting from toothbrushing on demineralized dentin may play a role in tubal occlusion by forming a smear layer on the dentin surface. This accounts for the significantly reduced permeability after the erosive-abrasive challenge for all groups when comparing sound and eroded dentin. According to the literature, reduction in dentin permeability ranges from 5 to 50% after treatment with different anti-erosive dentifrices^{17,36}. In the current study, permeability decreased by 14 to 45%, considering all evaluated toothpastes.

This study has some limitations, such as the fact that it did not identify the composition of the particles in the top of the dentinal tubules. The toothpastes evaluated contain other components besides fluoride compounds, which may influence the results. The current study chose to evaluate commercially available toothpastes in order to represent real-life conditions. Further *in situ* and *in vivo* studies evaluating the effectiveness of different toothpastes are needed to verify the influence of human saliva on eroded dentin, seeking to prolong the effects of toothpastes on dentinal tubule occlusion for the treatment of DH.

CONFLICT OF INTEREST DISCLOSURE

The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

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