

RESEARCH ARTICLE

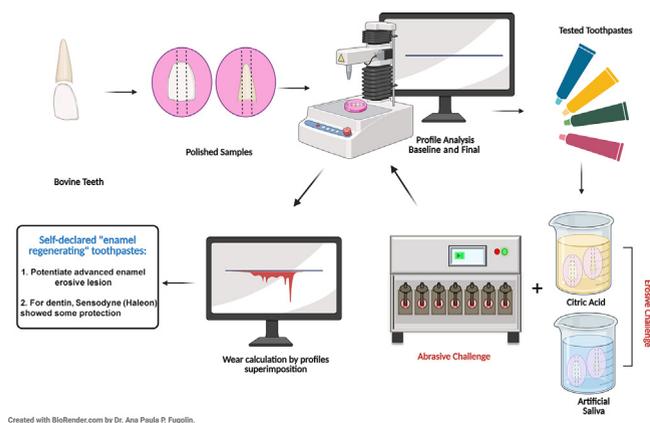
Effect of regenerating toothpastes on abrasion of eroded enamel and dentin

An in vitro study

Mariele Vertuan, PhD^a; Eduardo Lista Francisco^a; Monique Malta Francese, MSc^a; Júlia França da Silva, DDS^b; Ana Carolina Magalhães, PhD^a

^aDepartment of Biological Sciences, Bauru School of Dentistry, University of São Paulo, Bauru, São Paulo, Brazil;

^bDepartment of Surgery, Stomatology, Pathology and Radiology, Bauru School of Dentistry, University of São Paulo, Bauru, São Paulo, Brazil.



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Why Is This Important?

Erosive tooth wear is a growing oral health care concern, affecting a large portion of the global population. There is limited scientific evidence about commercial products that claim to regenerate tooth structure, specifically eroded tooth enamel. This study addresses a critical knowledge gap by evaluating the effect of commercially available toothpastes marketed as enamel regenerating on advanced erosive lesions. The findings showed that rather than offering protection, some of these products increased enamel and dentin wear compared with a fluoride toothpaste designed to control erosion (Elmex Erosion Protection; GABA International AG). For dentin, only 1 of the tested products showed a similar protective effect to that of the positive control. These results are important for both consumers and dental professionals. Although such products are popular and widely used, their claimed benefits may not accurately reflect their actual performance, especially in cases of advanced erosion. Understanding their limitations contributes to more informed decision making and promotes evidence-based recommendations. Although further in situ and clinical studies are needed, this research contributes to the broader discussion on how these preventive products can affect long-term oral health.

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Abstract

Background. This in vitro study evaluated the effect of self-declared enamel regenerating toothpastes on advanced erosive tooth lesions.

Methods. A total of 48 bovine crown and root samples were allocated into the toothpastes: Elmex Erosion Protection (GABA International AG, 1,400 ppm F; pH, 4.69) (positive control); Dentalclean Regenerator Sensitive (Dentalclean, 1,450 ppm F; pH, 4.97); Sensodyne Repair & Protect (GlaxoSmithKline) (1,426 ppm F; pH, 9.78) and erosion only (no abrasion) (negative control) (n = 12). All samples were subjected to erosive pH cycles (4 × 90 seconds/day in 0.1% citric acid, pH 2.5). Groups 1 through 3 underwent abrasive challenges (2 × 15 seconds/day abrasion + 45-second treatment) using toothpaste slurries and a brushing machine over 7 days. Between erosive and abrasive challenges, samples were immersed in artificial saliva. Wear was quantified using contact profilometry (in micrometers) to compare the initial and final profiles. Data were statistically compared using analysis of variance and Tukey test ($P < .05$).

Results. Brushing with Dentalclean (3.67 [0.47] μm) induced the greatest enamel wear (SD), followed by Sensodyne (2.99 [0.56] μm). Both were significantly different (SD) from Elmex (1.85 [0.38] μm) ($P < .0001$), which did not differ from erosion only (1.98 [0.57] μm). For

(Continued on next page)

dentin, brushing with Dentalclean (2.95 [0.60] μm) induced the greatest wear (SD), which was similar to that induced by erosion alone (2.87 [0.55] μm). In contrast, Elmex presented the lowest wear (SD) (1.26 [0.35] μm), similarly to Sensodyne (1.27 [0.58] μm).

Conclusions. Self-declared enamel regenerating toothpastes potentiate advanced erosive lesions. However, for dentin, Sensodyne toothpaste showed a similar result to the positive control.

Key Words. Erosion; fluoride; tooth erosion; toothpaste.

Introduction

Erosive tooth wear (ETW) is a multifactorial condition resulting from the exposure of dental substrates to nonbacterial acids, such as acids from diet and gastric sources.¹⁻⁴

This condition has drawn attention from researchers and clinicians worldwide because of its increasing prevalence and clinical detection rates.^{1,5,6} Estimates suggest that it affects approximately 30% through 50% of primary teeth and 20% through 45% of permanent teeth globally.^{7,8} It can also be associated with mechanical factors, such as abrasion, attrition, and abfraction,^{1,9} and accurately isolating each process has been challenged because of the complex interactions and dynamic nature of the oral cavity.¹

In enamel, ETW lesions are predominantly characterized by a substantial centripetal loss of mineralized tissue, leaving a partially demineralized surface layer with reduced microhardness and increased susceptibility to mechanical forces.¹⁰ In dentin, the lesion is more complex, because the exposure of the collagen matrix present in this tissue hinders ionic diffusion, delaying the progression of the lesion.^{11,12} In more severe cases, beyond esthetic concerns, patients may report symptoms such as tooth sensitivity, pain, and even functional impairment, all of which can negatively affect quality of life, especially when reaching deep dentin.^{1,13}

Among the preventive methods, the use of fluoride has proven effective in preventing erosive lesions¹⁴ by the creation of a mechanical barrier.⁷ Fluoridated toothpastes, especially those containing associated polyvalent metals, have also shown results in reducing the advanced erosive tooth lesions which simulate the ETW in vitro and in situ.¹⁵⁻¹⁷ Experimental toothpastes containing stannous fluoride plus sodium fluoride (NaF) and titanium tetrafluoride (TiF₄) plus NaF showed the potential to reduce advanced erosive lesions in enamel and dentin by approximately 65% through 70% compared with the placebo toothpaste.^{7,16-18}

Advanced erosive lesions in enamel cannot be regenerated, as the cells responsible for forming this tissue are no longer available in the mature enamel.¹⁹ On the one hand, dentin is formed by odontoblasts that line up along the dentin-pulp interface, in which they maintain the apposition of predentin and dentin throughout the life of a tooth.²⁰⁻²² Accordingly, there are toothpastes on the market advertised as enamel regenerating products, which in principle should stimulate the cells to produce dental tissues, according to their nomination. Evidence supporting a true

regenerative or even a protective effect against advanced erosive lesion development in enamel is limited.²³ On the other hand, although odontoblasts are able to respond to different agents, it is unlikely that the active components of these products reach them through dentin.

Like toothpastes that promise whitening effects,³ enamel regenerating toothpastes attract considerable media attention, leading many consumers to pay substantial amounts in search of solutions to prevent the esthetic and functional consequences of ETW. Thus, our study aimed to test commercially labeled enamel regenerating toothpastes with respect to their potential to reduce or not advance erosive tooth lesions compared with a commercial toothpaste indicated for ETW control. The null hypothesis is that the enamel regenerating toothpastes have similar potential to the commercial toothpaste indicated for ETW control in preventing eroded enamel and dentin wear.

Methods

Sample preparation

Our study was approved by the local Ethics Committee on the Use of Animals (016/2023).

Bovine crowns and roots were separated and secured individually in a prefabricated silicone mold, then embedded in autopolymerizing pink acrylic resin with the buccal surface exposed. After polymerization, the enamel (crown) and dentin (root) samples were polished using a metallographic polisher (Aropol 2V; Arotec) with silicon carbide sandpaper of 320, 600, and 1200 grit (Extex Corp). To prevent any abrasive interference on surface quality, the tooth on disk assemblies were rinsed in an ultrasonic device (Ultrasonic-T14; L&R Ultrasonic) at a frequency of 40 kHz for 2 minutes in deionized water.

The baseline profile was measured (using 5 readings with a 5 mm run and a distance of 250 mm between each reading) using a contact profilometer (MarSurf XCR 20; Mahr) in the mesiodistal direction of the sample. To enable accurate repositioning during subsequent profile readings, 2 scalpel blade marks were made to delineate the control and exposed surfaces, along with a small reference hole created with a one-quarter drill bit to mark the starting point. The system ensured the reproducibility of the x and y coordinates for each sample and the precision of the overlay between the

Table 1 Composition of toothpastes used in the experiment.*

Product (Trademark)	Composition
Elmex Erosion Protection (GABA International AG) Slurry pH: 4.69	Water, sodium fluoride (1,400 ppm F), glycerin, sorbitol, hydrated silica, hydroxyethyl cellulose, aroma, cocamidopropyl betaine, titanium dioxide, olaflur, sodium gluconate, stannous chloride, alumina, chitosan, sodium saccharin, potassium hydroxide, hydrochloric acid.
Dentalclean Regenerator Sensitive (Dentalclean) Slurry pH: 4.97	Water, sodium fluoride (1,450 ppm F), glycerin, silica, sorbitol, sodium lauryl sulfate, flavor, polyethylene glycol-12, cellulose gum, phosphoric acid, xylitol, sodium pyrophosphate, sodium saccharin, triclosan, menthol, mica, sodium benzoate.
Sensodyne Repair & Protect (GlaxoSmithKline) Slurry pH: 9.78	Sodium fluoride (1,426 ppm F), calcium sodium phosphosilicate, glycerol, macrogol, silicon dioxide, cocamidopropyl betaine, sodium methyl cocoyl taurate, flavor, titanium dioxide, carbomer, sodium saccharin.

*The toothpaste pH was measured in the slurry (1:3 water) by using a pH meter.

baseline and final profiles, allowing for accurate calculation of tooth wear.¹⁶

Then, two-thirds of the surfaces were protected with red nail polish, leaving 2 control surfaces, which were used for measuring and comparing the profiles.

Tested experimental groups

Enamel and dentin samples were randomly distributed in the experimental groups (12 per toothpaste group) (Table 1). The sample size was calculated based on previous studies,^{3,24,25} considering an α error of 5%, a β error of 20% and a difference (SD) of 3 (1) μm between the conventional (NaF-based toothpaste) and the most abrasive toothpaste (whitening toothpaste). Enamel regenerating toothpastes were selected based on their availability in our local main supermarkets and pharmacies.

pH cycling and abrasive challenge

The samples underwent a pH cycling for 7 days.^{3,24,25} It was performed 4 times per day as follows: immersion in 0.1% citric acid (pH 2.5) for 90 seconds (30 mL/sample) at 25 °C, rinsing in deionized water, and immersion in artificial saliva containing only minerals²⁶ for 2 hours in between (pH 6.8, 30 mL/sample) at 25 °C. The samples were further immersed in artificial saliva overnight, completing a daily 24-hour cycle.

After the first and the last acid exposure each day, treatment was performed using toothpaste slurry (1 toothpaste:3 water, 12 mL/sample) associated with abrasion, to simulate daily brushing twice a day.^{3,24,25} A brushing machine (Biopdi) was used, in which 12 manual brushes with a small head and extra-soft bristles (Curaprox 5460 UltraSoft; Curaprox) were attached at an angle of 12° to the direction of brushing, allowing a better contact area between the bristles and the tooth surface.

The toothbrush heads were aligned on the brushing machine brackets to ensure they remained parallel to the tooth surface. Each abrasive challenge was performed for 15 seconds, corresponding to approximately 45 back-and-forth movements (≈ 3 back-and-forth movements/second)^{3,16,24,25}

through reciprocal linear motion of the brushes, with the application of 150 g of weight on the center of the brush holder, to provide a force of 1.5 N on the samples at 37 °C.^{3,24,25} After the abrasion procedure, the samples remained in contact with the toothpaste slurry for a further 45 seconds, then were rinsed with deionized water (5 seconds) and stored in artificial saliva as previously described.

Wear calculation

To assess changes in the tooth surface profile, the cosmetic nail polish was removed using a 1:1 acetone-water solution, and 5 final readings were obtained from the same areas (x- and y-axis) as the baseline measurements. The final and baseline profiles were then aligned using MarSurf XCR 20 software to calculate wear (μm , limit of detection: 0.5 μm). The mean wear from the 5 readings per sample was calculated and used to compare the effect of the toothpastes on advanced erosive tooth lesion development.

Statistical analysis

GraphPad software Version 10 (GraphPad) was used for the statistical analysis. The normality and homogeneity were checked by Brown-Forsythe and Bartlett tests. Then, enamel and dentin wear means were compared by analysis of variance, followed by Tukey test. The level of significance adopted was set at 5%.

Results

Table 2 shows the mean (SD) of enamel and dentin wear (μm) promoted by the tested toothpastes on the eroded surfaces. For enamel, the erosion-only group showed lower wear values compared with the other toothpastes, except for Elmex Erosion Protection (GABA International AG), which served as the positive control group, showing the ability to reduce the effect of brushing on an eroded surface. Among the enamel regenerating toothpastes, Dentalclean Regenerator Sensitive (Dentalclean) showed the highest enamel wear, followed by

Table 2 Advanced erosive tooth lesion (μm) promoted by the tested toothpastes.*

Groups	Elmex Erosion Protection (GABA International AG), Mean (SD)	Dentalclean Regenerator Sensitive (Dentalclean), Mean (SD)	Sensodyne Repair & Protect (GlaxoSmithKline), Mean (SD)	Erosion Only, Mean (SD)
Enamel	1.85 (0.38) ^c	3.67 (0.47) ^a	2.99 (0.56) ^b	1.98 (0.57) ^c
Dentin	1.26 (0.35) ^b	2.95 (0.60) ^a	1.27 (0.58) ^b	2.87 (0.55) ^a

*Statistical significance was tested using analysis of variance and Tukey tests ($P < .0001$). Different letters within the same line show significant differences between the toothpastes.

Sensodyne Repair & Protect (GlaxoSmithKline), which differed significantly from both positive and negative controls.

In the case of dentin, brushing with positive control Elmex Erosion Protection resulted in the lowest wear values, similarly to Sensodyne Repair & Protect, both of which reduced wear compared with the other groups ($P < .0001$). Brushing with Dentalclean Regenerator Sensitive resulted in the highest wear value ($P < .0001$), which was similar to the wear promoted by the erosion-only group. Therefore, none of the toothpastes increased dentin wear; instead, 2 of them reduced dentin wear compared with the negative control.

Discussion

Self-declared enamel regenerating toothpastes potentiate advanced erosive lesions, except for Sensodyne on eroded dentin. Therefore, the null hypothesis was rejected because the tested toothpastes, in general, provoked a higher level of tooth wear than the commercial toothpaste indicated for ETW control.

Erosive challenges alone caused the lowest enamel wear value compared with eroded samples belonging to the toothpaste groups, as there was no further action of the abrasive forces in the former group. In turn, this group was similar to the action of the positive control toothpaste (Elmex Erosion Protection) on eroded enamel, indicating that the positive control toothpaste was suitable for our study, as it effectively prevented enamel damage. In dentin, the abrasive effect was not evident on eroded surfaces, as was the case with enamel, which is in agreement with the literature.²⁷ Tooth abrasion is not capable of removing collagen fibrils in the demineralized organic layer but only crushes or compresses the demineralized organic layer when applied after erosive challenges.²⁷ Because there is no removal of dentin by abrasion, it is expected that products containing active agents can reduce dentin wear, as shown by the positive control (Elmex Erosion Protection), which had lower values compared with erosion only. Elmex Erosion Protection has been considered the reference standard toothpaste against advanced erosive tooth lesions by other authors as well.^{28,29}

The positive control toothpaste, Elmex Erosion Protection, despite containing silica as an abrasive, also contains fluoride, stannous, and chitosan in its composition. Chitosan

is a biopolymer derived from chitin that can interact electrostatically with the tooth structure and easily adsorb to the tooth, forming a protective layer.^{17,30,31} It has lubricating effects that can reduce friction between the toothpaste abrasives and the toothbrush and tooth structure.^{32,33} Stannous cation combined with fluoride ion can further create an acid-resistant barrier on the tooth surface, able to protect that area mechanically and to reduce the progression of advanced erosive tooth lesions.²⁸ Francese et al¹⁷ found that Elmex Erosion Protection was effective in minimizing advanced erosive tooth lesions in situ. A placebo control toothpaste was not included in our study because each commercial toothpaste has its own unique base components, making it impossible to produce a single placebo that would be suitable for all tested toothpastes.

Conversely, Dentalclean Regenerator Sensitive and Sensodyne Repair & Protect toothpastes resulted in increased enamel and dentin wear compared with the positive control, except for Sensodyne Repair & Protect, which significantly reduced dentin wear.

Dentalclean Regenerator Sensitive toothpaste contains silica and pyrophosphate, abrasive components that corroborate the progression of advanced erosive tooth lesion, and has a low pH, which justifies the high enamel and dentin wear values found for this toothpaste group.^{3,24,25,34} It also contains some antimicrobial components, but their effect is not applicable in our study model.

Sensodyne Repair & Protect has the highest pH value and contains calcium sodium phosphosilicate (a source of calcium and a bioactive glass also known as NovaMin technology) and a low-intensity abrasive, silicon dioxide, which makes this toothpaste less abrasive. Compared with the tested toothpastes discussed above, Sensodyne Repair & Protect caused less wear, but it did not reach the protective effect of the positive control for enamel. However, in contrast, for dentin, Sensodyne promoted less wear, similarly to the positive control, Elmex. This may be attributed to an interaction between the components of the toothpastes and the collagen fibrils, resulting in the formation of a precipitate, as reported by Athanasiadou et al.³⁵

Calcium sodium phosphosilicate undergoes several chemical reactions in aqueous solution, forming carbonated hydroxyapatite on the dentin surface. In addition to enhancing the action of NaF compared with other toothpastes, this property would provide sealing of the dentinal

tubules and, consequently, inhibition of dentin demineralization.³⁵ Then, the eroded dentin surface brushed with Sensodyne showed less wear than that observed in the eroded dentin only. This possible mechanism of action could be proven using methods such as scanning electron microscopy in the future.

Tooth development, also known as odontogenesis, is initiated by the interaction between 2 types of dental tissues, the dental epithelium and mesenchyme, mediated by several molecules and stimuli.^{36,37} Therapies to repair tooth damage and loss rely on the use of synthetic materials that are not able to rebuild the tooth structure.^{36,37} Despite some products containing different types of calcium phosphate sources, they do not provide all the conditions to stimulate tissue growth, especially in enamel, in which no cells are available. So far, the most effective finding of the toothpastes is the precipitation of a disorganized mineral layer on the tooth surface, which in turn presents low acid resistance.³⁸ When the products are applied during brushing, their positive action must be balanced with the damage effect provoked by the brush action and the toothpaste's abrasiveness, making the process more challenging. Therefore, attention should be paid to promote the better formation of organized apatite crystal layers and to minimize the abrasivity of toothpastes, making the precipitation more resistant to further chemical or mechanical removal.^{39,40}

In the literature, there are no comparative articles to our study, to our knowledge. Athanasiadou et al³⁵ investigated the chemical and ultrastructural changes of dentin after exposure to regenerating toothpastes (Dentalclean Regenerating Booster [Dentalclean], Sensodyne Repair & Protect). In their study, human dentin disks were subjected to 14 consecutive days of toothbrushing (twice per day) followed by agitation in artificial saliva. The samples were also subjected to an acidic challenge from 7 through 14 days. The microscopic analysis revealed precipitate deposition on dentin surfaces and formation of nanocrystalline hydroxyapatite within the dentinal tubules of the respective groups, which corroborates the results found in our study for Sensodyne toothpaste.

There is a limitation in identifying a specific reason for the differences between the toothpastes. Considering that we tested commercial toothpastes with different compositions, a combination of factors may have contributed to their potential to provoke or minimize tooth wear, such as pH, type, size, and quantity of abrasives, type and quantity of fluoride, and the presence of other components. Furthermore, in vitro models do not simulate certain salivary properties, such as the formation of acquired pellicle, buffering capacity, and phosphatase actions. In clinical situations, saliva is present during erosive and abrasive challenges, which were not simulated in our study, as the samples were exposed to the remineralizing solution only between the challenges. Another limitation of our study is that only the loss of surface structure was evaluated through the profilometry method. No in-depth analysis was performed regarding the

formation or deposition of elements and the morphology of the tooth surface by scanning electron microscopy.

Conclusions

These results must be verified using in situ models before advising patients to avoid using the tested toothpastes. On the basis of these results and study limitations, the tested self-declared enamel regenerating toothpastes, in general, instead of protecting, potentiate advanced erosive tooth lesions, compared with a commercial toothpaste applied to control erosive lesions under this model. For enamel, Dentalclean and Sensodyne toothpastes presented the highest wear values. However, for dentin, Sensodyne toothpaste was similar to the positive control (Elmex Erosion Protection) in reducing wear. More research needs to be performed to provide more information concerning the effect of commercial self-declared enamel regenerating toothpastes on eroded and healthy teeth.

Disclosure

None of the authors reported any disclosures.

Email acm@usp.br. Address correspondence to Dr Magalhães.

Dr Vertuan and Mr Francisco contributed equally to this article and should be considered co-first authors.

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ORCID Numbers. Eduardo Lista Francisco: <https://orcid.org/0000-0001-7701-0598>; Júlia França da Silva: <https://orcid.org/0000-0003-1314-8795>. For information regarding ORCID numbers, go to <http://orcid.org>.

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