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# Toothpaste factors related to dentine tubule occlusion and dentine protection against erosion and abrasion

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## Abstract

**Objectives** To investigate the effect of toothpastes on dentine surface loss and tubule occlusion, and the association of toothpaste-related factors to each of the outcomes.

**Materials and methods** One hundred and sixty human dentine specimens were randomly distributed into 10 groups, according to different toothpastes. The specimens were submitted to artificial saliva (60 min), citric acid (3 min), and brushing abrasion (25 s; totalizing 2 min in toothpaste slurries). This was repeated five times and two outcome variables were analyzed: dentine surface loss (dSL; μm) and tubule occlusion by measurement of the total area of open tubules (Area-OT; $\mu m^2$). Data were analyzed with Kruskal-Wallis and Mann-Whitney tests ($\alpha = 0.05$); bivariate and multivariate regressions were used to model the association of the chemical (pH, concentration of $F^-$, $Ca^{2+}$, and $PO_4^{3-}$ and presence of $Sn^{2+}$) and physical (% weight of solid particles, particle size, and wettability) factors of the toothpastes to both outcome variables.

**Results** Toothpastes caused different degrees of dSL and did not differ in Area-OT. All chemical and physical factors, except the presence of $Sn^{2+}$, were associated with dSL ($p < 0.001$). Area-OT was associated only with the presence of $Sn^{2+}$ ($p = 0.033$).

**Conclusion** Greater dSL was associated with lower pH, lower concentration of $F^-$, higher concentration of $Ca^{2+}$ and $PO_4^{3-}$, greater % weight of solid particles, smaller particle size, and lesser wettability, whereas tubule occlusion was associated with the presence of $Sn^{2+}$.

**Clinical relevance** Depending on their chemical and physical composition, toothpastes will cause different degrees of dentine tubule occlusion and dentine surface loss. This could, in turn, modulate dentine hypersensitivity.

**Keywords** Dentinal tubules · Dental erosion · Dental abrasion · Toothpaste · Anti-erosion · Desensitizing

## Introduction

The frequent contact of non-bacterial acids with the tooth surface causes dental erosion. It starts with the softening of the tooth surface that is less resistant to mechanical forces, leading to the progression of this condition, and to surface loss. Additionally, gingival recession can further result in the exposure of cement, which can be easily lost due to dental erosion and mechanical forces, exposing the underlying root dentine. This uncovered dentine will also demineralize when in contact with the acids, leaving the dentinal tubules patent [1]. Stimuli occurring on this dentine surface can then induce a pain response called dentine hypersensitivity [2].

Dentine hypersensitivity is characterized as a short and sharp pain in response to external stimuli, not related to any other defect or disease [3, 4], and can impact the quality of life of the patients [5]. Treatments for dentine hypersensitivity act either by occluding the opened dentinal tubules or directly on the pulp nerves, increasing the pain threshold [6]. The first treatment of choice for dentine hypersensitivity is the use of homecare products, such as toothpastes [7].

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Toothpastes are important for tooth cleaning and serve as a carrier for active ingredients for tooth protection. In this sense, toothpastes may have special claims, which are associated with its action on specific conditions, such as treatment of dentine hypersensitivity (desensitizing toothpastes) or protecting the tooth surfaces against dental erosion (anti-erosion toothpastes). Their active ingredients for the previous condition will act on tubule occlusion or nerve desensitization [6], and for the latter they will act mainly by forming an acid-resistant layer on the tooth surface [8]. As dental erosion is one of the main predisposed factors for dentine hypersensitivity, the prevention of the first reduces the occurrence of the second [4].

Despite the claim of the toothpastes, it is expected that they have protective effects and cause the least possible loss of dental hard tissues. A previous study analyzing toothpastes with desensitizing and/or anti-erosion claim on enamel showed different ranges of surface loss, regardless of their claim [9]. This study showed that the chemical and physical factors of the toothpaste slurries played an important role in the amount of enamel surface loss. Presence of stannous, higher concentration of $Ca^{2+}$ and $PO_4^{3-}$, higher % weight of solid particles, smaller particle size, and lower wettability were associated with lower enamel loss. However, the factors associated with dentine loss are still not well known. Moreover, since enamel and dentine have different characteristics and the progression of dental erosion differs on both substrates [10], the association of these factors with dentine loss will probably be different. Additionally, these factors may also occlude the dentine tubules. In view of this, with the present study, we sought to investigate the effect of desensitizing and/or anti-erosion toothpastes on dentine surface loss and tubule occlusion, analyzing the association of these outcomes to chemical and physical factors of the toothpaste slurries.

## Materials and methods

### Specimen preparation

One hundred and sixty dentine slabs (3 mm × 3 mm × 1 mm) were cut from the cervical area of human molar roots and embedded in acrylic resin. The experiment was carried out in accordance with the approved guidelines and regulations of the local ethics committee (Kantonale Ethikkommission: KEK), which categorized the teeth as "irreversibly anonymized" because they had been pooled, so no previous approval from the committee was necessary.

The specimens were ground flat with silicon carbide paper discs of decreasing grain size (18.3 μm to 5 μm) under constant water cooling (LabPol 21, Struers). Then, they were polished (LabPol 6, Struers), under constant cooling, for 60 s with felt and 3 μm grain diamond paste (DP-Stick P, Struers), finalizing by polishing a further 60 s with 1 μm grain diamond paste. Between the polishing procedures and at the end of the preparation, the specimens were rinsed and ultrasonic-cleaned in deionized water for 60 s. Two-thirds of the specimens' surfaces were protected with an adhesive tape leaving an area of 3 mm × 1 mm in the center exposed to the experimental cycles.

### Experimental groups

The specimens were randomly distributed into 10 groups, with 16 specimens per group (Table 1). This sample size was based in a previous study with the same experimental model [9]. Toothpastes with desensitizing and/or anti-erosion claim were tested and allocated according to their main claim. Artificial saliva (AS, the same as used for the slurry preparation) and a regular fluoridated toothpaste (Colgate Caries Protection) were used as negative control and reference toothpaste, respectively. Toothpaste slurries were prepared daily, right before the toothbrush abrasion, by mixing 25 g of toothpaste with 50 g of artificial saliva [9, 11].

Artificial saliva [1.25 mM $Ca(NO_3)_2 \cdot 4H_2O$, 0.90 mM $KH_2PO_4$, 129.91 mM KCl, 59.93 mM Tris buffer, and 2.2 g/l porcine gastric mucine; pH 7.4] [12] was prepared weekly and stored at − 20 °C. Before each cycle, a fresh aliquot of artificial saliva was thawed and used to incubate the specimens to prepare the toothpaste slurries and for brushing the group AS [11].

### Erosion-abrasion cycles

The specimens were submitted to a 5-day erosion-abrasion cycle, with 1 cycle/day, on the same model as performed previously on enamel [9]. In each cycle, the specimens were individually incubated in artificial saliva in a shaking water bath (60 min, 37 °C, 70 rpm, travel path 50 mm), followed by erosive challenge in 1% citric acid (pH 3.6, 3 min, 25 °C, 70 rpm, travel path 50 mm). Then the specimens were immersed in the fresh slurry or AS for 2 min (room temperature) during which time they were brushed for 25 s (50 toothbrush strokes, 2 N, 120 strokes/min, travel path 40 mm, 40 mm/s) with an automatic brushing machine (Zahnbürstmaschine, Syndicad Ingenieurbüro, Munich, Germany). After each procedure, the specimens were washed with deionized water and slightly dried with air. Between the experiments and overnight, the specimens were constantly kept in a humid chamber, at room temperature.

### Dentine surface loss

After 5-days of erosion-abrasion cycle, the adhesive tapes were removed from the surfaces and the dentine surface loss

**Table 1** Details of the experimental groups

| Groups | Active ingredients | Claim |
|---|---|---|
| Artificial Saliva (AS) | | Negative control |
| Colgate® Caries Protection[a] | 1450 ppm $F^-$ (MFP and NaF) | Reference |
| Sensodyne® Repair and Protect[b] | 1450 ppm $F^-$ (NaF)<br>Calcium sodium phosphosilicate | Desensitizing |
| elmex® Sensitive Professional[c] | 1450 ppm $F^-$ (MFP)<br>Arginine and calcium carbonate | Desensitizing |
| Sensodyne® Rapid Relief[d] | 1040 ppm $F^-$ (NaF)<br>Strontium acetate | Desensitizing |
| Blend-a-Med® Complete Protect Expert[e] | 1450 ppm $F^-$ ($SnF_2$ and NaF)<br>3436 ppm $Sn^{2+}$ | Desensitizing |
| elmex® Erosion Protection[c] | 1400 ppm $F^-$ (AmF and NaF)<br>3500 ppm $Sn^{2+}$<br>Chitosan (0.5%) | Anti-erosion |
| Sensodyne® Pronamel[d] | 1450 ppm $F^-$ (NaF)<br>Potassium nitrate | Anti-erosion |
| Candida® Protect Professional[f] | 1450 ppm $F^-$ (MFP)<br>Oligopeptide-104 | Anti-erosion |
| Regenerate®[g] | 1450 ppm $F^-$ (MFP)<br>Calcium silicate and sodium phosphate | Anti-erosion |

Manufacturer; country of acquisition
[a] Colgate-Palmolive; Switzerland
[b] GlaxoSmithKline; France
[c] Colgate-GABA; Switzerland
[d] GlaxoSmithKline; Switzerland
[e] Procter and Gamble; Austria
[f] Migros; Switzerland
[g] Unilever; France

(dSL) was analyzed using an optical profilometer (MicroProf 100, FRT the art of metrology, Germany). A central area of the specimen was scanned, including the reference areas and the treated area. Then, 5 lines within 0.2 mm distance were traced on the scan, comprising both reference and treated areas. These lines were individually analyzed with a software (FRT Mark III), which calculated the difference in height between the reference areas and the treated area. The mean of the analysis of the five lines corresponds to the dSL of each specimen (μm).

## Total area of open tubules

For assessing the effect of the toothpastes on tubule occlusion, 10 specimens from each group were randomly selected, covered with two layers of carbon and analyzed with scanning electron microscopy (SEM; FEI, Quanta FEG 650, Czech Republic), using an acceleration voltage of 5 kV and magnification of × 1500. The microscope images were obtained from the center of the treated area of each specimen.

An area of $3.75 \times 10^3$ $\mu m^2$ was analyzed using ImageJ software to obtain the total area of open tubules (Area-OT). The number of open tubules (Number-OT) was also recorded.

## Chemical and physical analyses of toothpaste slurries

Chemical factors, such as the pH, the presence of stannous ($Sn^{2+}$, according to the description of the manufacturer in the tube), the concentration of calcium ($Ca^{2+}$), phosphate ($PO_4^{3-}$) and fluoride ($F^-$), and physical factors, such as % weight of the solid particles and particle size of the toothpaste slurries were analyzed and associated with enamel surface loss in a previous study [9]. Here, we used the results of the previous study to analyze the association of these factors with dSL and Area-OT.

Wettability analysis of dentine with toothpaste slurries was performed with a drop shape contact angle device (Drop Shape Analysis System DSA 10 MK2, Krüss, Germany; needle ∅ = 1.1 mm, drop volume 1 μl), according to the previous study [9].

## Statistical analysis

Data of dSL and the Area-OT were statistically analyzed with Kruskal-Wallis and Mann-Whitney tests. The significant values were adjusted by Bonferroni correction for multiple tests.

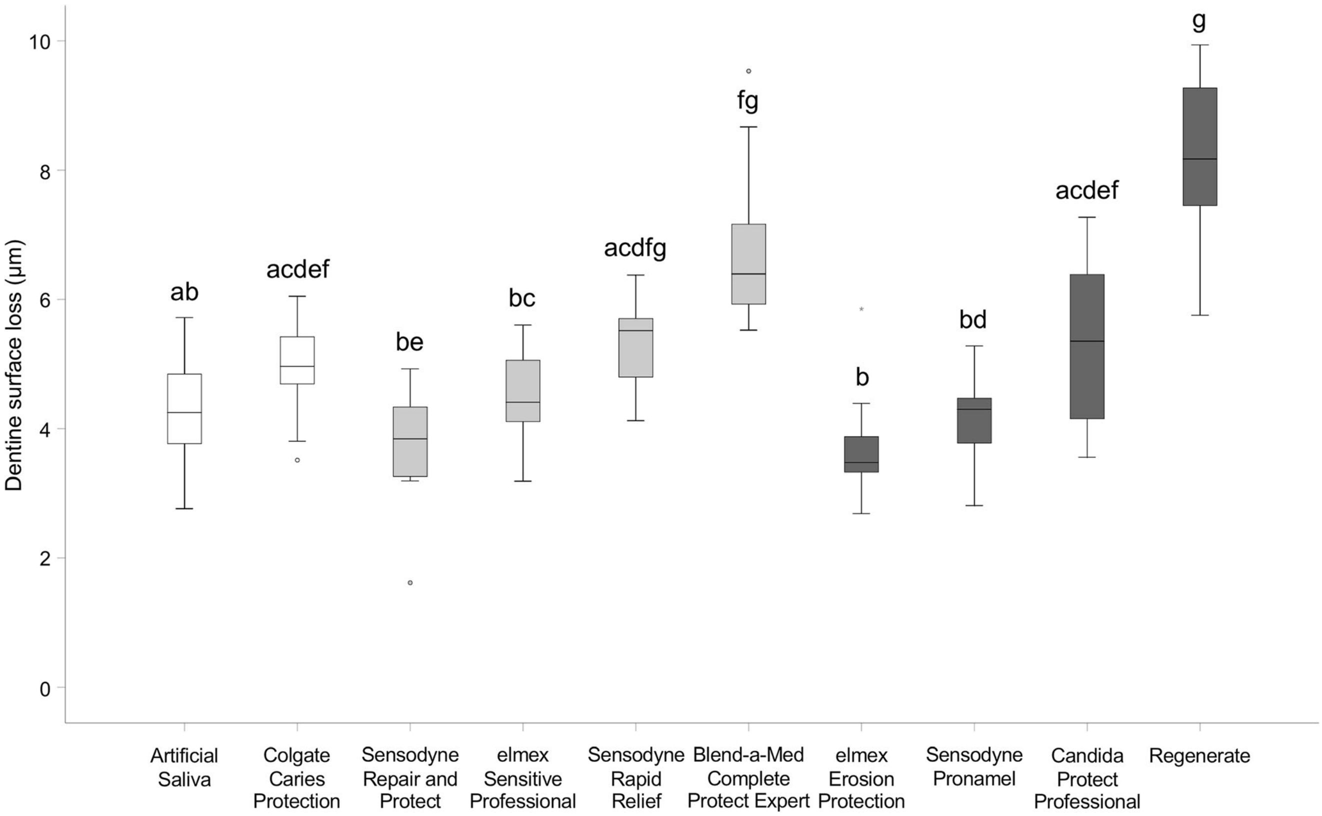


**Fig. 1** Dentine surface loss for the different experimental groups. Different colors represent the different claims of the toothpastes: white boxes—control groups, light gray boxes—desensitizing; dark gray boxes—anti-erosion. Different letters denote significant differences between the groups

The association of the chemical and physical factors with dSL and Area-OT was analyzed with general linear models. Bivariate regression analyses were performed considering each factor and each outcome variable (dSL and Area-OT). To analyze the interplay of the significant factors associated with each of the outcome variables, multivariate regression was performed. The factors with $p$ values < 0.20 in the bivariate models were included in the multivariate model using a backward stepwise approach. Variables with a $p$ value < 0.05 were retained in the final regression model [9]. The multivariate regressions were performed controlling for the dentine related factors (Number-OT, Area-OT, and dSL), which were retained in the model independent of their significance. Bivariate regression analyses were also performed to verify the association of the claim of the toothpastes with dSL and Area-OT.

All statistical analyses were performed using IBM SPSS Statistics version 22. Statistical significance was set at 5%.

## Results

All experimental groups presented different levels of dSL, with the median ranging from 3.44 to 8.20 µm, regardless of the claim of the toothpastes (Fig. 1). Low dSL values were observed for two desensitizing toothpastes (Sensodyne Repair and Protect and elmex Sensitive Professional) and two anti-erosion toothpastes (elmex Erosion Protection and Sensodyne Pronamel), which were neither different from each other nor from the control group AS ($p > 0.05$). One toothpaste of each claim (Blend-a-Med Complete Protect Expert and Regenerate) presented high dSL values, which were not different from each other ($p > 0.05$). Elmex Erosion Protection was the only toothpaste with low dSL that significantly differed from the reference toothpaste (Colgate Caries Protection; $p = 0.031$).

The median of Area-OT varied from 6.40 to 93.79 µm$^2$. The overall test showed significant differences between the groups ($p = 0.023$), but these differences were not significant in the pairwise analyses after correction of the $p$ values (Fig. 2).

The chemical and physical factors of toothpaste slurries and AS are in parts from João-Souza et al. [9], except for the wettability results that are for dentine (Table 2). The results of the regression analyses for dSL and for Area-OT are presented in Tables 3 and 4, respectively. Regarding dSL, the presence of $Sn^{2+}$ was the only factor of the toothpaste slurries that was not significantly associated. The other factors were significantly associated with dSL in both bivariate and multivariate analyses. For pH, the concentration of $Ca^{2+}$, wettability, and

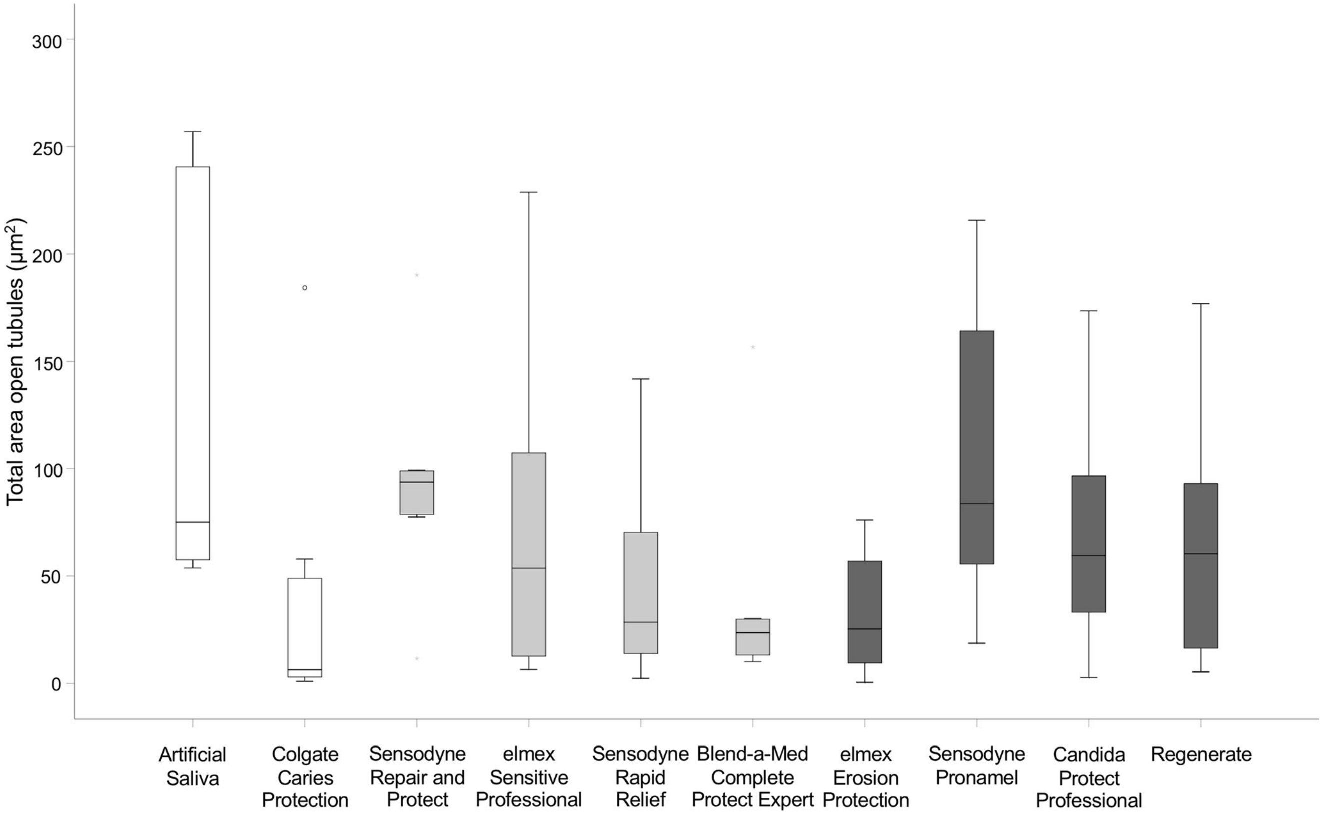


**Fig. 2** Total area of open tubules for the different experimental groups. Different colors represent the different claims of the toothpastes: white boxes—control groups, light gray boxes—desensitizing; dark gray boxes—anti-erosion. The groups were not significantly different

particle size, the direction of the association changed in the multivariate model in comparison to the bivariate analysis (Table 3). Considering all significant factors, lower dSL was associated with higher pH, lower concentration of $Ca^{2+}$ and $PO_4^{3-}$, higher concentration of $F^-$, lower % weight of solid particles, bigger particle size, and higher wettability of the toothpaste slurries. Regarding Area-OT, the final multivariate model showed that tubule occlusion (smaller Area-OT) was only associated with the presence of $Sn^{2+}$. No association was found between the claim of the toothpastes and dSL (estimate ± standard error − 0.224 ± 0.302, $p = 0.459$) or between the claim of the toothpastes and Area-OT (estimate ± standard error 2.189 ± 17.566, $p = 0.901$).

## Discussion

Desensitizing and/or anti-erosion toothpastes presented different levels of dSL independent of their claim and could not completely protect dentine against erosion and abrasion. This is in agreement with a study analyzing the effect of anti-erosion toothpastes on dSL, which likewise did not find a superior effect when compared to a fluoridated toothpaste [13]. Studies evaluating desensitizing toothpastes also showed different levels of dSL [14, 15].

For the AS group, the specimens were brushed only with artificial saliva, which does not contain any active ingredient or solid particles. The first could further protect the dentine against erosion, whereas the latter could add to the abrasion of the dentine surface. In this study, AS presented low dSL, and we regard this as the amount of abrasion caused purely by the toothbrush itself. Interestingly, all of the tested toothpastes contained some kind of active ingredients, which should protect the dentine surface, yet none of the toothpastes presented less dSL than AS. On the other hand, all toothpastes contained solid particles, which could further increase surface loss, but most of the toothpastes did not present more dSL than AS. Two possible explanations could be (1) that the protective effect of the active ingredients in the toothpaste slurries compensated for some of the abrasion effects of the solid particles; (2) the solid particles from these toothpastes could have had only a minor effect on the dentine, not promoting sufficient surface loss to be statistically different from AS in the present model.

Although different levels of dSL were observed between the groups, regarding Area-OT no difference was observed. Most of the desensitizing toothpastes act by tubule occlusion, due to deposits formed by their active ingredients on the dentine surface [16]. However, the abrasive particles can also have an occlusion effect by depositing on the entry of the

**Table 2** Median and interquartile range (IQR) of dentine related factors (dSL = dentine surface loss; Area-OT = total area of open tubules; Number-OT = number of open tubules); chemical and physical factors of the toothpaste slurries and artificial saliva (in parts from [9]; http://creativecommons.org/licenses/by/4.0/)

| | Dentine-related factors | | | Chemical factors | | | | | Physical factors | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Groups | dSL median (IQR) | Area-OT median (IQR) | Number-OT median (IQR) | pH | $[Ca^{2+}]$ (mmol/l) | $[PO_4^{3-}]$ (µmol/l) | $[F^-]$ (ppm) | Presence of $Sn^{2+}$ | % weight of solid particles | Drop shape (mean angle) | Particle size (µm) |
| Artificial Saliva | 4.17 (3.26–4.73) | 75.11 (56.60–244.62) | 39 (25–43) | 7.00 | 0.59 | $8.5 \times 10^{-3}$ | 0.07 | No | 0.0 | 21.8 | None |
| Colgate Caries Protection | 4.79 (4.44–5.21) | 6.40 (2.87–51.12) | 4 (3–32) | 6.89 | 0.23 | $4.8 \times 10^{-2}$ | 39.66 | No | 45.5 | 11.2 | < 50 |
| Sensodyne Repair and Protect | 3.53 (3.23–4.26) | 93.79 (77.53–99.21) | 38 (29–42) | 8.63 | 8.90 | $5.5 \times 10^{-1}$ | 245.10 | No | 27.6 | 22.5 | < 50 |
| elmex Sensitive Professional | 4.51 (3.88–5.07 | 53.66 (10.28–167.99) | 29 (9–46) | 8.75 | 0.29 | 6.0 | 62.09 | No | 40.8 | 13.7 | < 20 |
| Sensodyne Rapid Relief | 5.32 (4.80–5.59) | 28.60 (11.83–95.03) | 20 (10–42) | 6.52 | 0.52 | $7.4 \times 10^{-4}$ | 96.44 | No | 28.0 | 18.9 | ≥ 50 |
| Blend-a-Med Complete Protect Expert | 6.54 (5.94–7.58) | 23.67 (12.03–30.16) | 18 (10–30) | 5.57 | 0.18 | $3.0 \times 10^{-4}$ | 311.10 | Yes | 34.7 | 16.9 | ≥ 50 |
| elmex Erosion Protection | 3.44 (3.32–4.34) | 25.44 (5.55–64.33) | 22 (5–40) | 4.70 | 0.62 | $8.4 \times 10^{-8}$ | 253.20 | Yes | 26.9 | 15.9 | < 50 |
| Sensodyne Pronamel | 4.13 (3.78–4.37) | 83.86 (39.92–164.42) | 42 (19–51) | 7.03 | 0.09 | $7.2 \times 10^{-3}$ | 311.65 | No | 25.3 | 29.5 | < 50 |
| Candida Protect Professional | 5.68 (4.46–6.49) | 59.51 (7.62–121.42) | 31 (10–36) | 6.91 | 2.27 | $2.8 \times 10^{-2}$ | 25.20 | No | 31.0 | 19.1 | < 50 |
| Regenerate | 8.20 (7.57–9.27) | 60.35 (14.89–100.27) | 38 (10–53) | 9.02 | 0.08 | 66.1 | 0.72 | No | 34.7 | 11.9 | ≥ 50 |

**Table 3** Association between dentine surface loss and the different chemical and physical factors

| Independent variable | Bivariate model* | | Multivariate model† | |
|---|---|---|---|---|
| | Estimate ± SE | *p* value | Estimate ± SE | *p* value |
| Dentine-related factors§ | | | | |
| Number of open tubules | 0.011 ± 0.010 | 0.268 | 0.024 ± 0.007 | 0.001 |
| Total area of open tubules | − 0.002 ± 0.002 | 0.497 | − 0.003 ± 0.002 | 0.064 |
| Chemical factors | | | | |
| pH | 0.216 ± 0.094 | 0.022 | − 3.155 ± 0.551 | < 0.001 |
| $Ca^{2+}$ concentration | − 0.179 ± 0.047 | < 0.001 | 0.845 ± 0.160 | < 0.001 |
| $PO_4^{3-}$ concentration | 0.051 ± 0.005 | < 0.001 | 0.177 ± 0.026 | < 0.001 |
| $F^-$ concentration | − 0.003 ± 0.001 | 0.002 | − 0.014 ± 0.003 | < 0.001 |
| Presence of $Sn^{2+}$ | | | | |
| Not present‡ | 0 | | | |
| $Sn^{2+}$ present | 0.147 ± 0.314 | 0.640 | | |
| Physical factors | | | | |
| % weight of solid particles | 0.037 ± 0.011 | < 0.001 | 0.465 ± 0.073 | < 0.001 |
| Drop shape (angle) | − 0.105 ± 0.014 | < 0.001 | 0.694 ± 0.121 | < 0.001 |
| Particle size | | | | |
| No particles‡ | 0 | | 0 | |
| ≤ 20 μm | 0.190 ± 0.412 | 0.644 | − 7.311 ± 1.258 | < 0.001 |
| 20 to 50 μm | 0.086 ± 0.319 | 0.788 | − 12.465 ± 2.060 | < 0.001 |
| ≥ 50 μm | 2.451 ± 0.336 | < 0.001 | − 10.000 ± 1.911 | < 0.001 |

*Analyses of each independent variable with the outcome (dSL) variable

† Only variables with a *p* value < 0.2 in the bivariate model were considered for the multivariate analysis, and the variables were only kept in the multivariate model if *p* value < 0.05;

§ The multivariate model was made controlling for these variables; due to their inherent association with the dentine, they were kept in the model independent of their significance;

‡ Reference category

*SE* standard error of the estimate

tubules [17]. This may explain the lack of difference of Area-OT between the toothpastes, regardless of their claim. Nevertheless, AS did not contain any solid particles that would occlude the dentinal tubules. In spite of that, AS was not different from the other groups. We can speculate that the abrasion caused by the toothbrush itself on the demineralized dentine also played a role on tubule occlusion, by forming smear layer on the dentine surface, even in the absence of toothpaste slurry. Another important point that could also explain the lack of difference between the groups is regarding the last experimental step, which was toothbrush abrasion. Once we applied the toothpastes, the deposits were formed on the dentine surface, independently of the toothpaste used. These deposits could, however, have different susceptibilities to acid demineralization. So, if our experimental model had ended with an acid challenge, we speculate that it would have been possible to observe some differences in tubule occlusion between the groups, perhaps in line with the surface loss data.

To better understand the effect of toothpastes on dSL and tubule occlusion, their chemical and physical factors were investigated. One main factor is fluoride, whose protective effect against dental erosion is generally attributed to the formation of a calcium fluoride-like ($CaF_2$) layer, which increases in view of a lower pH and a higher fluoride concentration [18]. In the present study, we used slurries with fluoride concentrations ranging up to 311 ppm, due to the dilution factors. Such low concentrations would probably have little protective effects on dentine, since fluoride has a limited protective effect on dentine, even in increased concentrations [8, 19–21]. Despite having observed that higher $F^-$ concentrations were associated with lower dSL, we still agree with the cited studies and speculate that fluoride might bring some preventive effect particularly when in association with polyvalent ions and some polymers [8, 18, 22, 23], but any effect of fluoride alone will be minimal.

**Table 4** Association between total area of open tubules and the different chemical and physical factors

| Independent variable | Bivariate model* | | Multivariate model† | |
|---|---|---|---|---|
| | Estimate ± SE | $p$ value | Estimate ± SE | $p$ value |
| Dentine-related factors§ | | | | |
| Number of open tubules | 3.029 ± 0.292 | < 0.001 | 3.051 ± 0.278 | < 0.001 |
| Dentine surface loss | − 3.454 ± 5.087 | 0.497 | − 7.258 ± 3.178 | 0.022 |
| Chemical factors | | | | |
| pH | 13.875 ± 6.015 | 0.021 | | |
| $Ca^{2+}$ concentration | 3.013 ± 3.328 | 0.365 | | |
| $PO_4^{3-}$ concentration | 0.001 ± 0.416 | 0.998 | | |
| F − concentration | − 0.030 ± 0.066 | 0.759 | | |
| Presence of $Sn^{2+}$ | | | | |
| Not present‡ | 0 | | 0 | |
| $Sn^{2+}$ present | − 42.168 ± 19.882 | 0.034 | − 26.978 ± 12.687 | 0.033 |
| Physical factors | | | | |
| %weight of solid particles | − 1.504 ± 0.731 | 0.040 | | |
| Drop shape (angle) | 3.032 ± 1.454 | 0.037 | | |
| Particle size | | | | |
| No particles‡ | 0 | | | |
| ≤ 20 μm | − 22.959 ± 36.743 | 0.532 | | |
| 20 to 50 μm | − 62.248 ± 30.472 | 0.041 | | |
| ≥ 50 μm | − 76.240 ± 31.693 | 0.016 | | |

*Analyses of each independent variable with the outcome (total area open tubules) variable

† Only variables with a $p$ value < 0.2 in the bivariate model were considered for the multivariate analysis, and the variables were only kept in the multivariate model if $p$ value < 0.05

§ The multivariate model was made controlling for these variables; due to their inherent association with the dentine, they were kept in the model independent of their significance

‡ Reference category

*SE* = standard error of the estimate

The pH of solutions is important for their protective effect against erosion and abrasion [18]. The same is true for toothpastes, as their active ingredients require specific pH values in order to exert their desired effect. A lower pH increases the formation of $CaF_2$-like deposits on the tooth surfaces and is also important for the mechanism of action of other active ingredients, such as stannous [18, 24]. However, our results showed that higher pH was associated with lower dSL. The range of pH in toothpaste slurries varied from 4.70 to 9.02, and the toothpastes containing stannous were the ones with lower pH values. The lack of association of the presence of stannous to dSL would, in turn, mean that low pH is also not associated with dSL; whereas the higher pH values of all other toothpastes will have a greater influence on dSL, thus explaining the relationship between pH and dSL.

The use of stannous-containing oral-care products has shown increased protection of dental surfaces when compared to the use of only fluoridated products, especially when formulated as a mouth rinse solution [25–27]. However, in the present study, no association of stannous and dSL was observed. One reason for this may be the limited protective effect of stannous-containing toothpastes when applied with toothbrush abrasion [21]. Nevertheless, the toothpaste that presented low dSL and significantly differentiated from regular fluoridated toothpaste is the one that has stannous in its formulation (elmex Erosion Protection). This result is probably due to other factors and ingredients of the toothpaste. This toothpaste showed low % weight of solid particles and particles as big as 50 μm, which were associated with low dSL. Furthermore, the concentration of free $F^-$ measured in its slurry is high (~ 250 ppm), in comparison to the other toothpastes. Besides, it contains chitosan that might interact with collagen, by forming cross-links, protecting the dentine against enzymatic degradation [28]. Regarding Area-OT, tubule occlusion was only associated with the presence of $Sn^{2+}$. Therefore, although not having shown a protective effect against dental erosion and abrasion, stannous did play a role in reducing the area of the dentinal tubules. This is in accordance with other studies that also showed tubule occlusion with the use of stannous-containing toothpastes [29, 30].

Calcium and phosphate are important for the equilibrium between the oral cavity and the dental hard tissue [10]. Thus, higher concentrations of these ions in oral care products would raise their availability on the tooth surface. While this increases the protection regarding dental caries, it cannot be directly inferred to erosion, because remineralization in this case is limited [1, 31]. Moreover, the use of toothpastes containing calcium compounds provided no further protection for dentine, when compared to fluoridated toothpaste [32]. Actually, in the present study, higher concentration of $Ca^{2+}$ and $PO_4^{3-}$ was associated with greater dSL.

The relative dentin abrasivity (RDA) is a common used measurement for classifying the abrasivity of the toothpastes [33]. However, in the present study, RDA of the toothpastes was not analyzed. Instead, we analyzed the % weight and the size of the solid particles of the toothpastes. This was done because these factors impact directly the RDA values and because the regression analyses require independent factors. Moreover, the RDA is measured on sound dentine, while in the present study, erosive challenges were performed, which can modulate the abrasivity of the toothpastes [33].

When particle size was individually analyzed, lower dSL was associated with smaller particle size. However, when considering all the significant factors together in the regression analysis, the direction of the association changed, and lower dSL was associated with bigger particle size. This change may be a reflex of the great dSL observed for the toothpastes that presented the biggest particles. Taking into consideration that lower dSL was also associated with lower % weight of solid particles and higher wettability, we can speculate that bigger particles would be easier taken away from the dentine surface during the toothbrush abrasion.

The wettability analysis showed that a higher wettability of the dentine surface by the toothpaste slurries decreases its loss. As a higher wettability means an easier spreading of the liquid on the surface, this effect might be due to an increased contact of the active ingredients of the toothpaste with the dentine surface, increasing its protection against the next erosive challenge. Nevertheless, more studies are necessary to better understand the role of wettability of toothpastes on the protection against dental erosion and abrasion.

Considering the claim of the toothpastes, they were not associated neither with dSL nor with Area-OT. These results reflect the different levels of dSL regardless of the toothpaste claim and the lack of difference between the groups in relation to the Area-OT. However, it should be kept in mind that regardless of the active ingredients and of claim of the toothpastes, they should not increase dSL. Regarding the Area-OT, more investigations on the role of toothbrush abrasion is needed.

We can conclude that the toothpastes caused different degrees of dSL, but not differences on tubule occlusion. Lower dSL was associated with higher concentration of $F^{-}$, higher pH, lower concentration of $Ca^{2+}$ and $PO_4^{3-}$, lower % weight of solid particles, bigger particle size, and higher wettability. Tubule occlusion was only associated with the presence of $Sn^{2+}$. The claim of the toothpastes was not associated with neither dSL nor Area-OT.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All procedures performed in this study were in accordance with the ethical standards of the local research committee (Kantonale Ethikkommission: KEK) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study was granted exemption from requiring ethics approval, as detailed in the "Materials and methods" section.

**Informed consent** For this type of study, formal written consent is not required.

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