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ORIGINAL ARTICLE

Efficacy of toothpastes in the prevention of erosive tooth wear in permanent and deciduous teeth

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Abstract

Aim To evaluate the erosive preventive effect of toothpastes in permanent (PT) and deciduous teeth (dt).

Design Enamel samples were divided into five groups ($n = 20$): G1: placebo toothpaste; G2: NaF toothpaste; G3: AmF-NaF-$SnCl_2$ anti-erosion toothpaste; G4: SnF_2–toothpaste; and G5: NaF anti-erosion toothpaste for children. The samples were exposed to five erosion-abrasion cycles (artificial saliva incubation; 3 min in 1% citric acid; 2 min in slurry, toothbrush abrasion, 50 strokes, 200 g). Surface microhardness (SMH), surface specular reflection intensity (SRI), and cumulative surface loss (CSL) were measured. Comparisons among toothpastes were evaluated using Kruskal-Wallis tests and comparisons between PT and dt were evaluated using Wilcoxon's rank sum test.

Results G1 exhibited significantly lower SMH values in PT than the other toothpastes ($p < 0.05$), with no significant differences among the others groups. In dt, G1 and G4 exhibited significantly different values than the other groups ($p < 0.05$). G4 exhibited lower values of SRI in both types of teeth. Deciduous teeth presented significantly higher SRI than PT ($p < 0.05$), except for G3. Deciduous teeth generally presented higher CSL than PT, except for G3.

Conclusions Deciduous teeth were more prone to mineral loss than permanent teeth. G5 exhibited better efficacy for both teeth, while G3 exhibited a better preventive effect only for deciduous teeth.

Clinical relevance Erosive tooth wear prevalence in children is growing and deciduous teeth are more susceptible than permanent teeth. Considering this, it is important to know the preventive effect of different toothpastes in an initial erosion-abrasion model.

Keywords Erosive tooth wear · Permanent teeth · Deciduous teeth · Tooth erosion · Tooth wear · Toothpaste

Introduction

Erosive tooth wear (ETW) is defined as the loss of tooth substance by mechanical and chemical processes not involving bacteria [1]. The most important sources are dietary and gastric acids [2–5]. ETW is increasingly recognized as a common condition in pediatric dentistry; its prevalence has been reported to range from 10 to 80%, mostly diagnosed in its initial stages, and increases significantly with age [6, 7].

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ETW in deciduous teeth should not be considered a short-term physiological process but rather a predictive indicator of future wear in permanent dentition [8, 9]. The management of ETW in children should include an early diagnosis, an evaluation of different etiological factors, risk identification, and a proposal of preventive measures to delay the progression of the condition, once surface loss is irreversible [8, 10].

With regard to ETW prevention, the benefits of toothpaste compounds containing fluoride are greater than the adverse effects, such as abrasivity. Various active ingredients have been tested with respect to their ability to form acid-resistant precipitates on dental surfaces [11]. When compared to non-fluoride toothpastes, fluoride-containing toothpastes have better preventive effects against ETW. However, in order to develop toothpastes that promote the formation of more acid-resistant precipitates, other formulations have been tested using amine or stannous fluoride compounds, as well as phosphates or biopolymer additives [12].

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Ganss et al. [13] evaluated the effect of commercial toothpastes that claim to provide anti-erosive effects compared to conventional fluoride toothpastes. Conventional NaF toothpastes were able to reduce erosive tissue loss even in severe erosive conditions but had limited efficacy with respect to toothbrushing abrasion. The formulations that claimed anti-erosive effects were not superior; however, tin-containing toothpaste had promising anti-erosion potential, which was counteracted by abrasion effects [13].

The preventive effect of different formulations of toothpastes, mouth rinses, and varnishes with respect to ETW has been tested in several studies, most of them in permanent teeth [13–16]. However, some considerable differences between permanent and deciduous teeth should be addressed. The enamel of deciduous teeth is less mineralized, and its surface hardness and elasticity are lower than permanent teeth. Therefore, it could be assumed that deciduous teeth are more susceptible to acidic dissolution than permanent teeth [8].

The outermost layer of aprismatic enamel and the absence of perikymata in deciduous teeth make the diagnosis of tooth wear in its early stages difficult in daily practice [8]. Optical assessment of ETW has been tested to improve diagnosis. The mode of action of these devices is based on the fact that abrasion of eroded teeth results in the partial removal of the softened enamel tissue and an increase in the specular reflection intensity due to smoothing of the etched enamel surfaces [17].

In laboratory studies, early stages of enamel and dentin dissolution can be determined through surface hardness measurements. Detection of enamel abrasion is also possible by calculating the depth of the indentations, so long as the lowest point of the indentation is not changed and is not removed by abrasion. Only the surrounding tissue is removed, and the surface loss can be calculated based on the change in the indentation length [18].

Considering the presumed difference between deciduous and permanent enamel, and the lack of information about the preventive effect of some commercial toothpastes with respect to ETW on deciduous teeth, the aim of the present study is to evaluate the preventive effect of different toothpastes in an initial erosion-abrasion model. The null hypotheses tested were that permanent and deciduous teeth are similar with respect to decreases in surface microhardness (SMH), variations in specular reflection intensity (SRI), and calculated surface loss (CSL) and that preventive effect of toothpastes tested is similar.

Materials and methods

Experimental design and groups

The sample size calculation was based on the difference in calculated surface loss for different toothpaste treatments from a previous study and considered a balanced one-way analysis of variance power calculation (level of significance = 0.05, 80% power); it indicated a minimum number of 16 in each group [10]. In order to have extra samples for scanning electron microscopy (SEM) imaging, and counting with losses possible during the experiment, 20 samples were randomly selected for each group.

Sample preparation

Sound premolars (n = 100) and deciduous molars (n = 100) were randomly selected from a pool of extracted teeth. The present experiment was carried out in accordance with the approved guidelines and regulations of the local ethical committee (Kantonale Ethikkommission: KEK). The crowns were separated from the roots (Isomet® Low Speed Saw, Buehler, Düsseldorf, Germany), cross-sectioned into lingual and buccal sides, and embedded into resin (Paladur, Heraeus Kulzer GmbH, Hanau, Germany). Under constant water cooling, the samples were abraded (LabPol 21, Struers, Copenhagen, Denmark) with silicon carbide paper discs (grain size 1000, 2400, 4000) for 30 s for each grain size. Next, samples were polished for 60 s with 3 μm diamond abrasive under constant cooling (LaboPol-6, DP-Mol Polishing, DP-Stick HQ, Struers, Copenhagen, Denmark). After this procedure, a 200-μm layer of the surface enamel was removed. All samples were stored in a mineral solution [19] and underwent an additional polishing step with 1-μm diamond abrasive paste immediately prior to the beginning of the experimental phase.

Toothpastes

Five different toothpastes were tested in this study (Table 1). Toothpaste slurries were prepared daily by mixing 20 g of toothpaste with 40 g of artificial saliva ($CaCl_2 2H_2O$; KH_2PO_4; NaCl; KCl; Mucin—gastric mucin from porcine stomach, type II) [20]. The pH of the slurries was measured using a pH meter (691 Ph Meter, Metrohm, Herisau, Switzerland). The wettability was also evaluated using a drop-shape analysis system (DSA 10 Mk2, Krüss GmbH, Hamburg, Germany). Five drops of each toothpaste slurry were made, and the contact angle was measured.

Erosion-abrasion cycles

The samples were exposed to five erosion-abrasion cycles. Prior to erosive challenge, the samples were incubated in artificial saliva [20] at 37 °C for 1 h, dipped in deionized water three times, and gently dried with oil-free air. Initial SRI and SMH measurements were then performed.

The erosive challenge consisted of sample immersion for 3 min in 30 mL of 1% citric acid (pH 3.6) at 25 °C under

Table 1 Description of groups and toothpastes used in the study

Group	Composition	Active ingredient	RDA values	Slurries pH	Wettability drop shape	Brand/manufacturer
G1—negative control	Acqua, sorbitol, hydrated silica, hydroxyethyl cellulose, PEG 40, aroma, CI 77891, saccharin, Nipagin M, potassium hydroxide	None	80–100	6.47	49.5	Placebo toothpaste (GABA—Colgate)
G2—NaF toothpaste Positive control	Sorbitol, water, hydrated silica, sodium lauryl sulfate, trisodium phosphate, flavor, sodium phosphate, cellulose gum, carbomer, sodium saccharin, titanium dioxide, blue 1	NaF (1500 ppm)	120	6.84	19.0	Crest® (P&G)
G3—AmF-NaF-$SnCl_2$ anti-erosion toothpaste	Aqua, glycerin, sorbitol, hydrated silica, hydroxyethyl cellulose, aroma, cocamidopropyl betaine, CI 77891, olaflur, sodium gluconate, alumina, chitosan, sodium saccharin, potassium hydroxide, hydrochloric acid	NaF/AmF/$SnCl_2$ (1400 ppm)	40–60	4.77	30.5	elmex Erosion Protection® (GABA—Colgate)
G4—SnF_2 toothpaste	Glycerin, PEG-8, hydrated silica, pentasodium triphosphate, aroma, sodium lauryl sulfate, titanium dioxide, carbomer, sodium saccharin, stannous fluoride, cocamidopropyl betaine, limonene	SnF_2 anhydrous (1100 ppm)	119	6.62	0.0	Sensodyne Repair&Protect® (GSK)
G5—NaF anti-erosion toothpaste for children	Aqua, sorbitol, hydrated silica, glycerin, PEG-6, cocamidopropyl betaine, xanthan gum, aroma, sodium fluoride, sodium saccharin, sucralose, titanium dioxide, sodium hydroxide, limonene	NaF (1450 ppm)	30–40	6.68	54.0	Sensodyne ProNamel for children® (GSK)

constant agitation (70 rpm). The samples were washed and dried, and SRI and SMH measurements were repeated.

Afterwards, the samples were submitted to abrasion by immersing each sample in a toothpaste slurry for 2 min; during this time, the sample was brushed in the brushing machine (50 strokes; 200 g force). After each abrasive challenge, surface loss was calculated and final SRI and SMH measurements were performed.

Surface microhardness measurements

SMH Knoop measurements were performed (50 g/10s, UHL VMHT Microhardness Tester, UHL Technischer Mikroskopie, Aßlar, Germany). The percentage difference in SMH (ΔSMH) was calculated using the formula $\Delta SMH = SMH_f / SMH_0 \times 100$, where SMH_0 is the initial SMH and SMH_f is the value obtained after the fifth SMH measurement taken after the last erosion-abrasion cycle.

Enamel specular reflection intensity measurements

Enamel SRI measurements were carried out using a hand-held optical reflectometer [21]. The reflectometer tip was placed directly onto the enamel sample surface and was then manually inclined at different angles until the point of highest reflection intensity was registered [21]. The percentage difference in SRI (ΔSRI) was calculated using the formula $\Delta SRI = SRIf / SRI_0 \times 100$, where SRI_0 is the initial SRI (measured at baseline) and SRI*f* is the value obtained after the fifth SRI measurement taken after the last erosion/abrasion cycle.

Calculated enamel surface loss

A set of six Knoop indentations was made on the enamel surface. The lengths of these indentations were measured after the erosive cycles and re-measured immediately after the abrasive cycles [22]. Using these length values (L), each indentation depth (D) was calculated according to the equation $D = L / 2 \cdot \tan \alpha$, where $\alpha = 3.75°$, a constant parameter of the diamond indenter. Prior to the abrasive challenge, the indentations were longer (and therefore deeper) than after the abrasion. The difference in indentation depth before and after the toothpaste abrasion treatment was calculated for each indentation (calculated enamel surface loss), and this value represented the amount of enamel abraded away from the indentation surroundings [10]. The cumulative surface loss corresponded to the sum of each calculated enamel surface loss value during the five erosion-abrasion cycles.

Scanning electron microscopy analyses

Two samples from each group were randomly selected and sputtered with gold (Baltec MED 020, 20 mA, 0.015 mbar, in argon atmosphere). The samples were taken to the SEM chamber (JSM-6010 PLUS/LV SEM, JEOL, Tokyo, Japan) for analysis. SEM images of the enamel surfaces were taken at ×1000 and ×4000 magnifications.

Statistical analyses

Initially, all the data were checked by Shapiro-Wilk test for normality. Based on these preliminary analyses, with non-normal distribution of all variables, non-parametric tests were selected. Non-parametric two-way ANOVA was used to analyze the interaction of factors (time, toothpaste, and type of tooth). In order to test for time as a factor in the setting of repeated measures, a non-parametric ANOVA for longitudinal data was also performed. Simultaneous comparisons between more than two groups were done performing Kruskal-Wallis tests, comparisons between two groups using Wilcoxon's rank sum test. The level of significance was set to 0.05. All statistical results were calculated with R (versions 3.1.0 and 3.2.2).

Results

Surface microhardness measurements

A significant decrease in SMH values was observed throughout the experiment in both permanent and deciduous teeth for all toothpaste groups ($p < 0.001$). On interaction factors analysis both time and tooth type had a significant effect on SMH. The greater reduction in SMH values occurred after the first erosive challenge; after the abrasive challenges, the SMH values increased. In permanent teeth, the placebo toothpaste caused significantly greater enamel softening after the fifth cycle. All fluoride toothpastes caused significantly less enamel softening than the placebo toothpaste group ($p < 0.001$), but they were not significantly different from each other (Table 2). In deciduous teeth, the placebo toothpaste also exhibited a greater change in SMH values. Throughout the experiment, all fluoride toothpastes were significantly different from the placebo toothpaste with respect to SMH values ($p < 0.001$), but at the end of the experiment, the NaF anti-erosion toothpaste for children led to a decrease in SMH values that was not significantly different from the placebo toothpaste ($p = 0.116$) (Table 2).

To compare permanent and deciduous teeth, ΔSMH was calculated, once deciduous teeth exhibited lower SMH values than permanent teeth at baseline (KHN 358 ± 1.35 and 390 ± 7.55, respectively, $p < 0.05$). Deciduous teeth exhibited a greater decrease in ΔSMH, which was significantly different from permanent teeth in all toothpastes tested ($p < 0.05$) (Fig. 1).

Table 2 Surface microhardness and surface reflection index values, initial and final measurements, of permanent and deciduous teeth, of all groups tested

Group	Surface microhardness				Surface reflection index			
	Permanent teeth		Deciduous teeth		Permanent teeth		Deciduous teeth	
	Initial[a] Median (IQR)	Final Median (IQR)	Initial[a] Median (IQR)	Final Median (IQR)	Initial[a] Median (IQR)	Final Median (IQR)	Initial[a] Median (IQR)	Final Median (IQR)
G1—negative control	396.58 (26.25)	261.33[a] (44.67)	357.92 (34.5)	235.33[c] (40.16)	58.37 (6.01)	6.07[a] (1.83)	59.60 (5.95)	7.03[a] (1.85)
G2—NaF toothpaste Positive control	395.00 (30.17)	318.92[b] (35.67)	359.67 (33.75)	285.42[d] (35.15)	57.06 (8.63)	6.98[b] (4.34)	55.69 (9.18)	6.17[a] (4.13)
G3—AmF-NaF-$SnCl_2$ anti-erosion toothpaste	390.92 (31.66)	311.00[b] (51.50)	354.67 (16.05)	291.92[d] (28.75)	56.74 (6.03)	7.58[b] (5.85)	57.31 (6.89)	11.14[b] (4.74)
G4—SnF_2 toothpaste	381.42 (31.82)	306.92[b] (64.49)	358.58 (27.83)	279.58[d] (32.00)	53.97 (6.43)	3.90[c] (1.23)	54.74 (6.97)	3.70[c] (2.00)
G5—NaF anti-erosion toothpaste for children	404.67 (34.33)	305.25[b] (56.41)	363.67 (32.57)	247.08[c] (37.57)	59.73 (2.92)	6.66[a] (2.35)	59.07 (4.44)	7.01[a] (2.48)

Median (IQR) ($n = 20$ per group). Kruskal-Wallis tests. Different small letters at same column indicates statistically significant differences among groups

[a] Initial SMH and SRI values were similar in all groups and were statistically significant different to final values between all groups, in permanent and deciduous teeth

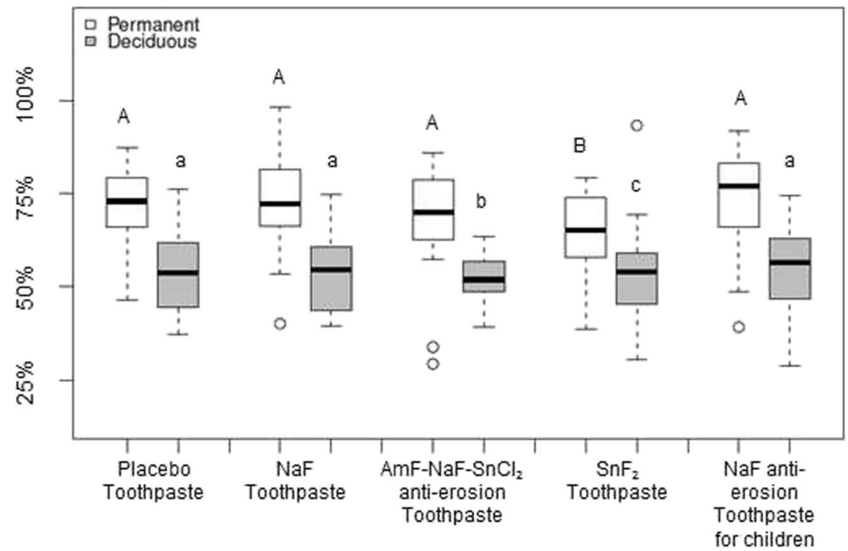

Fig. 1 Percentage difference in SMH (ΔSMH) after erosive-abrasive challenges in permanent and deciduous teeth, according to the different groups ($n = 20$). Statistically significant differences are shown with different letters: lowercase letters represent deciduous teeth; uppercase letters represent permanent teeth

Enamel specular reflection intensity measurements

In both permanent and deciduous teeth, the five toothpastes were significantly different from each other at the last SRI measurement. On interaction factors, analysis of both time and tooth type had a significant effect on SRI. Deciduous teeth had higher SRI values than permanent teeth (Table 2).

When comparing the ΔSRI in permanent teeth, only the AmF-NaF-$SnCl_2$ anti-erosion toothpaste presented lower and significantly different values ($p < 0.001$) compared to all other toothpastes. In deciduous teeth, both the SnF_2 toothpaste and the NaF anti-erosion toothpaste for children exhibited significantly different values compared to all other toothpastes ($p < 0.001$). When comparing the ΔSRI between permanent and deciduous teeth, no significant differences were observed between any toothpastes tested ($p > 0.05$, Fig. 2).

Calculated enamel surface loss

It was possible to detect significant differences in surface loss among all groups, from the first cycle on, for both permanent and deciduous teeth ($p < 0.001$). After each cycle, there was a progressive increase in surface loss. In permanent teeth, the NaF toothpaste led to more surface loss compared to all other toothpastes ($p < 0.001$). At the end of fifth cycle, placebo and NaF anti-erosion for children toothpastes exhibited significantly less surface loss than the other groups ($p < 0.001$) (Fig. 3a). In deciduous teeth, the NaF and SnF_2 toothpastes exhibited the highest surface loss values, which were significantly different from the other toothpastes at the end of fifth cycle ($p < 0.001$, Fig. 3b).

Deciduous teeth exhibited significantly more surface loss than permanent teeth during the entire experiment in four groups ($p < 0.001$). The only exception was the AmF-NaF-$SnCl_2$ anti-erosion toothpaste. This toothpaste led to similar surface loss in both deciduous and permanent teeth ($p > 0.05$).

SEM images

On SEM images (Fig. 4), the effect of erosion/abrasion cycles on enamel surface of permanent and deciduous teeth could be observed. In both types of teeth, AmF-NaF-$SnCl_2$ anti-erosion, SnF_2, and NaF anti-erosion for children toothpastes were better able to preserve the prismatic structure than placebo and NaF toothpastes. The enamel surface in the AmF-NaF-$SnCl_2$ anti-erosion toothpaste group showed less signs of erosive demineralization. No distinguishing characteristics could be observed between permanent and deciduous teeth at either lower (×400) or higher magnification (×1000).

Discussion

There are a variety of products, especially toothpastes, that claim to provide ETW protection, but most of them have been tested on permanent teeth. To our knowledge, this is the first study to investigate the anti-erosion effect of toothpastes on deciduous teeth.

Studies have shown controversial results with respect to whether deciduous teeth are more susceptible to ETW than permanent teeth [8]. In the present study, deciduous teeth presented lower initial SMH than permanent teeth. Moreover, when treated with non-fluoride-containing placebo toothpaste, deciduous teeth exhibited significantly greater softening than permanent teeth. On the other hand, no significant differences were observed between the two types of teeth when fluoride toothpastes were used.

The variation in SMH during the erosion-abrasion cycles in both permanent and deciduous teeth was similar to that

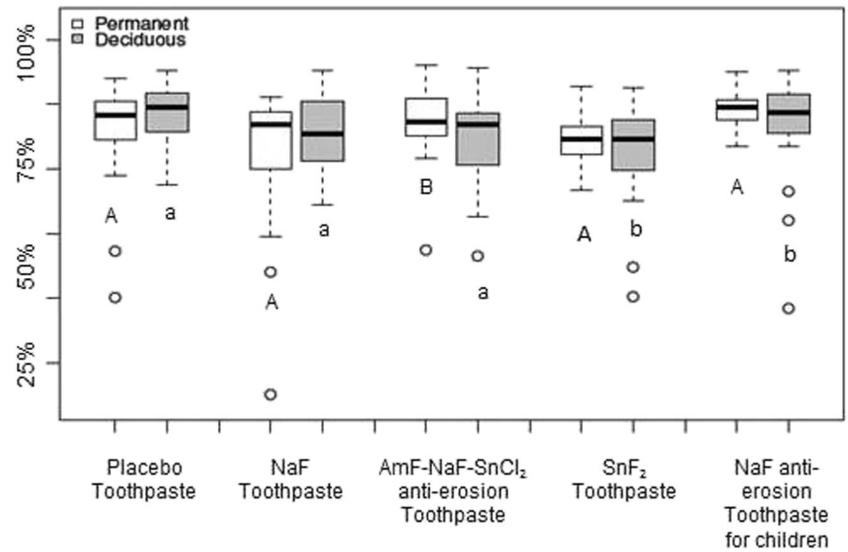

Fig. 2 Percentage difference in SRI (ΔSRI) after erosive-abrasive challenges in permanent and deciduous teeth, according to the different groups ($n = 20$). Statistically significant differences are shown with different letters: lowercase letters represent deciduous teeth; uppercase letters represent permanent teeth

reported in a previous study [10]. The greatest SMH decrease occurred during the first erosive challenge, and after each abrasive challenge, a small increase in SMH was observed. This is due to the fact that enamel surface was softened after erosive challenge, and this outermost softened layer was removed by tooth brushing during abrasive challenge. The treatment with different toothpastes was also responsible for the different decreases in SMH; as expected, placebo toothpaste group exhibited a greater decrease compared to fluoride toothpaste groups [10]. Therefore, fluoride toothpastes were found to significantly protect both permanent and deciduous teeth compared to placebo toothpaste.

Not only does the acid cause a decrease in surface hardness after an exposure, but it can also lead to the production of a rougher enamel surface. This roughened surface causes a decrease in surface reflection, as observed in the SRI results [24]. The subsequent abrasive challenge then partially removes this softened layer, leaving a slightly smoother enamel surface [17]. This was also observed in the SRI results, where an increase-decrease pattern was observed consistent with previous in vitro studies [22–24]. This evaluation is complementary to surface hardness measurement, and the comparison between SRI values of permanent and deciduous teeth were performed for the first time on this study. When considering the *SRI* values, both permanent and deciduous teeth treated with SnF_2 toothpaste showed significantly lower *SRI* values than the other groups. This toothpaste, originally indicated by the manufacturer for dentine sensitivity treatment, produced a different wear pattern on the enamel surface, as observed on SEM images, thus possibly leading to the lower *SRI* values found.

The main variable of this study, calculated surface loss, was measured through a modified surface hardness measurement,

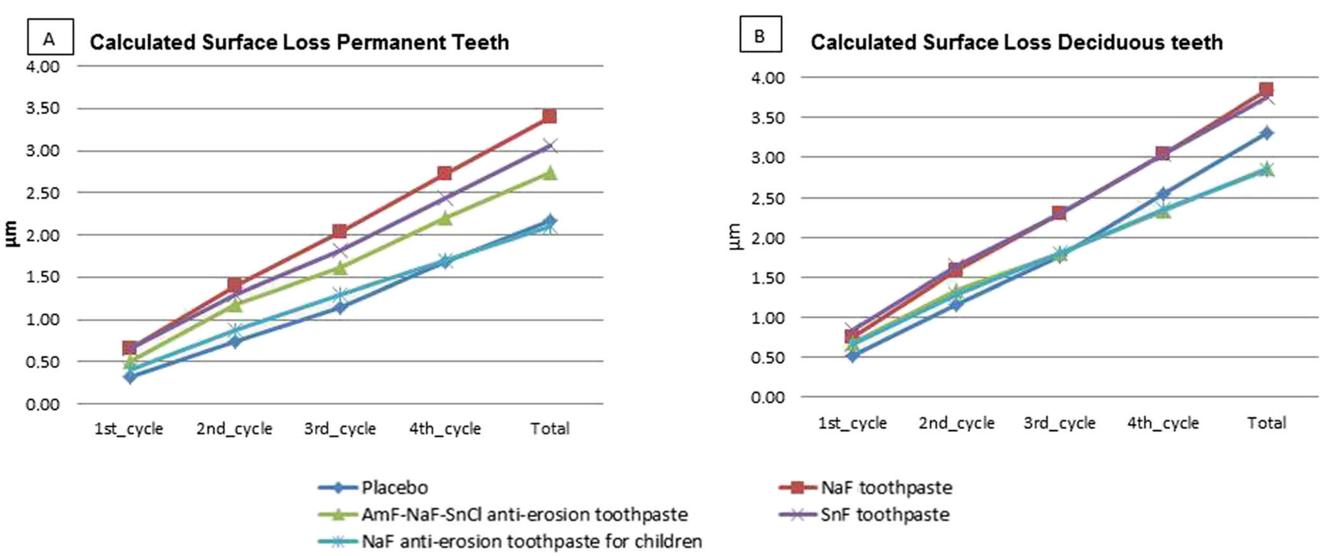

Fig. 3 Calculated surface loss during the erosion-abrasion cycles in permanent (**a**) and deciduous teeth (**b**), according to the different groups ($n = 20$)

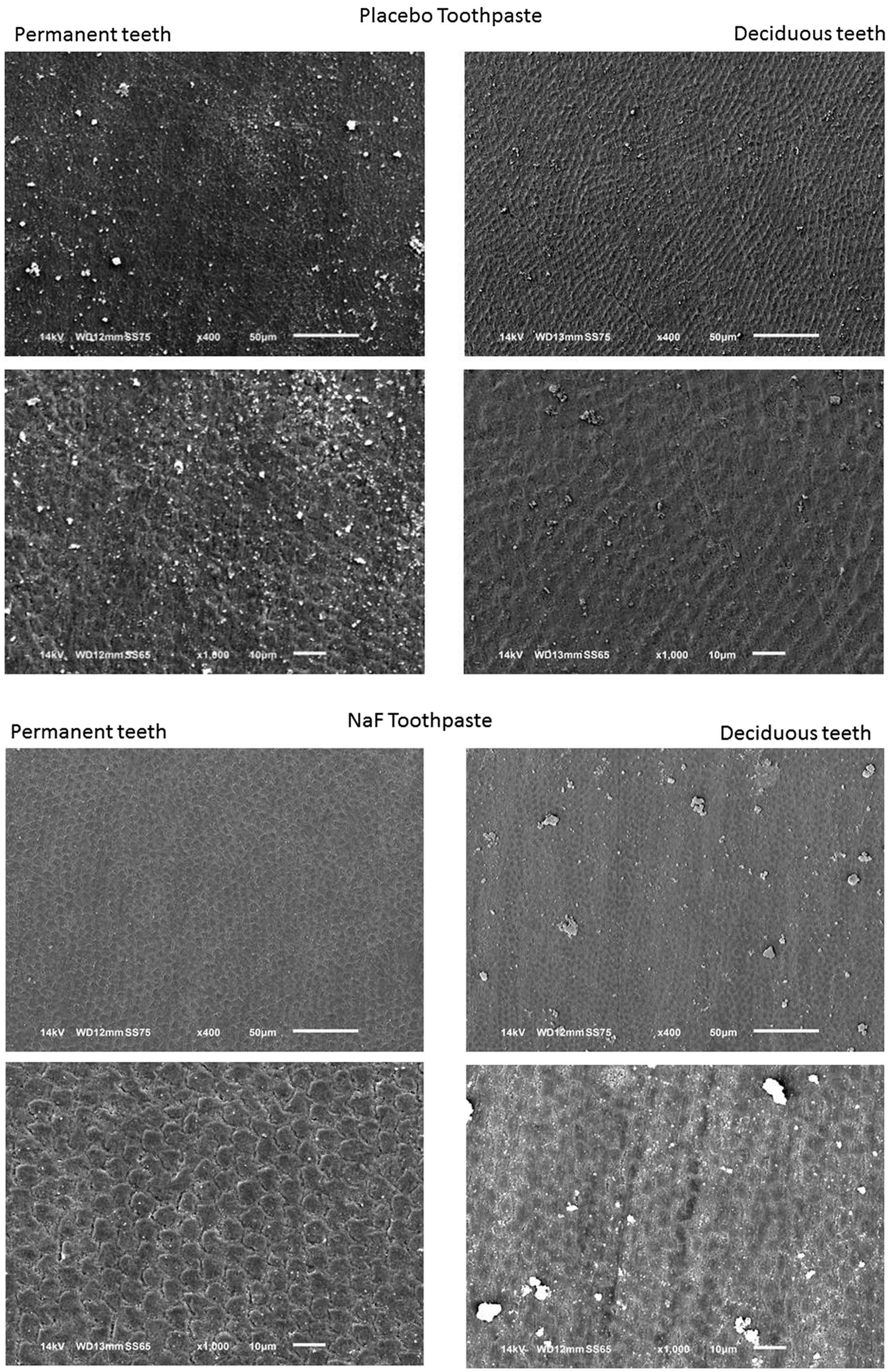

Fig. 4 SEM images of permanent and deciduous teeth after five erosion/abrasion cycles, according to toothpaste groups at lower (×400) and higher (×1000) magnifications

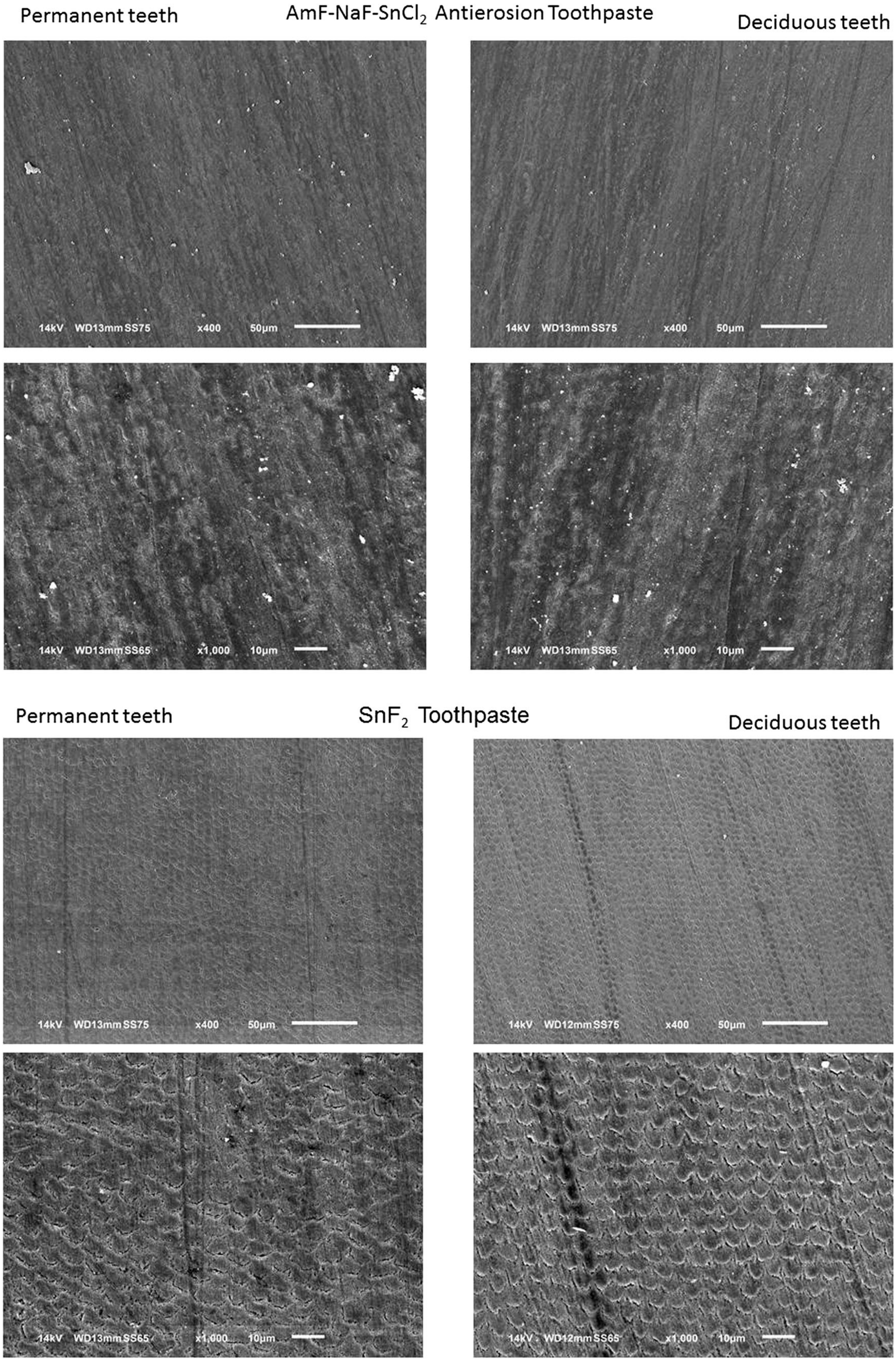

Fig. 4 (continued)

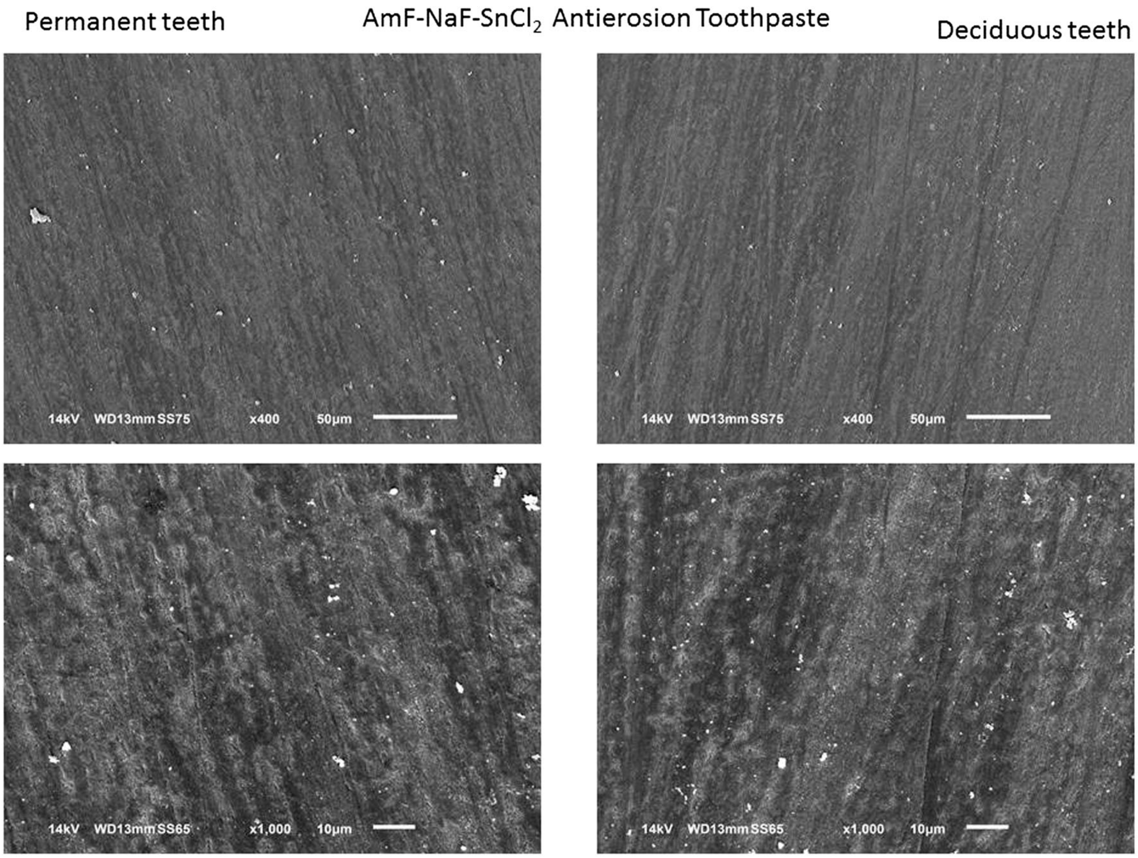

Fig. 4 (continued)

used in previous studies [10, 22]. This method was compared to focus variation 3D microscopy and contact stylus profilometry. All methods presented a linear pattern of surface loss measurements throughout the experiment, leading overall to a strong, statistically significant correlation between the methods, which presented consistent results for surface loss measurement [25]. Enamel surface loss was observed in all samples, and none of the tested toothpastes were able to fully prevent ETW. The NaF and SnF_2 toothpaste groups exhibited greater surface loss in both permanent and deciduous teeth compared to the other toothpastes. In deciduous teeth, treatment with anti-erosion toothpastes (AmF-NaF-$SnCl_2$ anti-erosion and NaF anti-erosion toothpaste for children) led to the least amount of enamel surface loss, statistically significantly different from other groups. In permanent teeth, the NaF anti-erosion toothpaste for children exhibited lower and statistically significantly different surface loss, except compared to the placebo toothpaste. The lowest Relative Dentine Abrasivity (RDA) and the highest wettability values, compared to other toothpastes of this study, could explain the better preventive effect on surface loss of NaF anti-erosion toothpaste for children. Since the placebo toothpaste had no active ingredients (F- or Sn^{2+}), it was expected that this toothpaste would lead to greater SMH decreases and, hence, greater surface loss. Surprisingly, despite the greater surface softening observed on samples treated with the placebo toothpaste, this toothpaste exhibited remarkably low enamel surface loss values for permanent teeth (2.16 μm) and for deciduous teeth (3.31 μm). The low enamel surface loss could be explained by the complex composition of the toothpastes, which despite the lack of active ingredients (fluoride and stannous) presented a better preventive effect.

In our study, the AmF-NaF-$SnCl_2$ anti-erosion toothpaste (G3) had the lowest surface loss values in deciduous teeth. This is in accordance with previous studies [10, 13, 26–28]. The Sn-containing salts form a more resistant layer on the enamel surface [14], and it can interact with the salivary pellicle [29] or be incorporated into the demineralized enamel surface [14]. The presence of chitosan as a thickener could also had improved the preventive effect of AmF-NaF-$SnCl_2$ anti-erosion toothpaste. Remarkably, this toothpaste showed similar surface loss in deciduous teeth as the NaF anti-erosion toothpaste for children (G5). Although the mode of action of the Sn-containing toothpaste was meticulously outlined by Schlueter et al. [14], the authors have mostly focused on permanent teeth. However, further studies are still necessary to fully elucidate the interaction of Sn_2+ with deciduous enamel.

The daily use of toothpastes is the main vehicle for the application of active ingredients, mainly fluorides and stannous compounds. These active ingredients should increase the acid resistance of tooth surfaces and pellicles, leading to lower tooth wear [8]. Nevertheless, toothpastes also contain excipients and thickeners that could interfere on wettability. In this study, different wettability values were measured in toothpaste slurries. The toothpastes which presented the lowest values, NaF and SnF_2 toothpastes (19.0 and 0.0, respectively), also presented higher cumulative surface loss, in permanent and deciduous teeth. This fact could signalize that wettability could also interfere on toothpaste erosion-abrasion preventive effect.

Nevertheless, toothpastes also contain abrasives, which could counteract the positive effects of the active ingredients [12]. Toothpaste abrasivity is usually measured according to RDA values, which are mostly a measure of dentine susceptibility and they do not quite reflect enamel susceptibility. Moreover, even Relative Enamel Abrasivity (REA) values do not fully represent the rate of enamel wear in an erosion-abrasion experiment. In the study by Philpotts et al. [30], the authors tested different toothpastes that presented different RDA and REA values and showed that these values do not always correlate [30]. In this case, even though the RDA values of the toothpastes used in this study were obtained from the manufacturers, the RDA values may not necessarily indicate the rate of enamel wear. The fact that RDA and REA values may not represent enamel wear rates was also observed in the study by Pickles et al. [31].

Other studies, however, have shown that toothpaste abrasivity can play a major role in the wear rate of acid-softened enamel. In the study by Wiegand et al. [32], the authors showed that abrasive-free toothpaste caused significantly less tissue loss than toothpastes with medium and high abrasivity, but the amount of enamel wear was generally similar for the two toothpastes with medium and high REA values [32]. Hara et al. [33] also presented similar results, and, additionally, the authors also showed that fluoride-containing toothpastes promoted significantly less abrasion than toothpastes without fluoride [33]. Strikingly, in the present study, we observed that the placebo toothpaste caused significantly less enamel wear than the other toothpastes, especially in permanent teeth.

The difference between the present study and the abovementioned studies is that the two other studies exposed samples to far more erosive and abrasive conditions in their experimental setups [32, 33]. This difference between the severity of erosive and (most importantly) abrasive challenges could account for the differences in the results. In the study by Hara et al., the number of toothbrush strokes per cycle (500 strokes) was probably enough to remove the entire softened enamel layer. In this study, there is agreement with Carvalho and Lussi [10], where the mode of action of the fluoride toothpastes is instead related to a partial inhibition of enamel softening during the subsequent erosive challenge, and the fluoride itself is not able to prevent substance loss when the softened enamel is brushed away [10].

The scenario is, however, different when less toothbrush strokes are used. If the samples were brushed for less time or with fewer strokes, not all of the softened layer will be removed. Some active ingredients, such as SnF_2, need repeated applications to build a resistance barrier to acid. In this initial erosion-abrasion model, we suggest that the active ingredients do not play as significant a role in prevention. Furthermore, since not all of softened layer was removed in this experiment (it was used only 50 toothbrush strokes per experimental cycle), it can be suggested that the amount of enamel loss was more dependent on the abrasivity of the toothpaste.

The role of toothpaste active ingredients and abrasives had been evaluated in some recent studies [34, 35]. There was a non-linear association between abrasiveness and amount of particles in a formulation, although the particle size had no impact. The particulate fraction could have an important action on toothpaste preventive effect in erosion/abrasion protocols [34]. Different active ingredients (amine fluoride, Sn_2F, and NaF) and chitosan had been tested with different amounts of abrasive silica, in slurries or with brushing. The amount of abrasives had no effect when toothpastes were applied as slurries but lead to more surface loss when applied with brushing. Chitosan added to Sn_2F formulation presented the better preventive effect, when applied as slurry [35]. Abrasives may counteract the efficacy of anti-erosion toothpastes either due to physical effects or due to interaction with active agents. In clinical situations, it is difficult to apply toothpaste without brushing. More studies should be done to overcome this side effect, together with the development of different formulations, as mousses or gel, which could dismiss brushing for application.

The SEM images helped to illustrate the different patterns of erosive tooth wear among the five groups. The placebo toothpaste led to a more disorganized surface, which indicates a more severe effect from the acid demineralization. The AmF-NaF-$SnCl_2$ anti-erosion and NaF anti-erosion for children toothpastes led to a more intact tooth structure, with the enamel prisms presenting a more defined contour.

The toothpaste compounds associated with fluoride, or the presence of fluoride in the toothpastes tested, did not appear to be the key factors underlying the different results found among the groups. Other toothpaste properties such as RDA, REA, wettability, and viscosity should be investigated in future studies to clarify the effect on erosive tooth wear.

Deciduous teeth were more prone to surface loss than permanent teeth. NaF anti-erosion toothpaste for children exhibited better efficacy for both permanent and deciduous teeth, while AmF-NaF-$SnCl_2$ anti-erosion toothpaste exhibited a better preventive effect only for deciduous teeth with regard to the main variable evaluated in this study, the calculated surface loss.

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Compliance with ethical standards

Conflict of interest Assunção CM declares that he has no conflict of interest. Lussi A declares that he has no conflict of interest. Rodrigues JA declares that he has no conflict of interest. Carvalho TS declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors. The present experiment was carried out in accordance with the approved guidelines and regulations of the local ethical committee (Kantonale Ethikkommission: KEK).

Informed consent The responsible for the children were informed about the use of the teeth for research purposes and informed consent was obtained from all individual participants included in the study.

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