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ORIGINAL ARTICLE

Effects of dentifrices differing in fluoride compounds on artificial enamel caries lesions in vitro

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Abstract The aim of this study was to compare the caries-preventive effect of a stabilized stannous fluoride/sodium fluoride dentifrice containing sodium hexametaphosphate with those of a regular, solely sodium fluoride-containing and amine fluoride-containing dentifrice on pre-demineralized bovine enamel specimens using a pH-cycling model. Bovine enamel specimens with two artificial lesions each were prepared. Baseline mineral loss of both lesions was analyzed using transversal microradiography (TMR). Eighty-five specimens with a mean (SD) baseline mineral loss of 3393 (683) vol% × µm were selected and randomly allocated to five groups ($n = 13/15$). Treatments during pH-cycling (28 days and 2×20 min demineralization/day) were: brushing twice daily with slurries of AmF (1400 ppm F^-), NaF (1450 ppm F^-), SnF_2/NaF (1100 ppm F^-/350 ppm F^-), and fluoride-free (FF) dentifrices or they were immersed in distilled water and remained unbrushed (NB). Subsequently, from each specimen one lesion was covered with acid-resistant varnish, while the remaining lesion was demineralized for another 14 days. Differences in integrated mineral loss ($\Delta\Delta Z$) were calculated between values before and after pH-cycling ($\Delta\Delta Z_{E1}$) as well as before pH-cycling and after second demineralization ($\Delta\Delta Z_{E2}$) using TMR. Treatments AmF and NaF induced a significantly higher mineral gain ($\Delta\Delta Z_{E1}/\Delta\Delta Z_{E2}$) compared to treatments FF and NB ($p < 0.05$; ANOVA test). Except for treatments AmF and NaF no significant differences in mineral loss between before and after pH-cycling could be observed ($p < 0.05$; t test) [$\Delta\Delta Z_{E1}$: AmF:1563 (767); NaF:1222 (1246); SnF_2/NaF:258 (1259); FF:−52 (1223); NB:−151 (834)]. Both dentifrices with either AmF or NaF promoted remineralization, whereas SnF_2/NaF dentifrice did not promote remineralization in a biofilm-free pH-cycling model.

Keywords Demineralization · Stannous fluoride · Amine fluoride · Sodium fluoride · In vitro

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Introduction

A variety of clinical studies could demonstrate the benefits, safety and cost-effectiveness of various means of fluoride delivery [1]. Dentifrices, one of the most widely practiced and effective ways to deliver free or soluble fluoride, are probably the most common products in healthcare [2]. Several fluoride compounds can be utilized intending to provide numerous different benefits. Especially metal ions such as stannous combine different therapeutic effects to control caries [3] as well as oral malodor [4]. The first stannous fluoride (SnF_2) dentifrices were combined with calcium pyrophosphate, sodium metaphosphate or other abrasive systems [5] in their formulations and provided up to 22–25 % reduction in dental caries compared to placebo

dentifrices [3]. As the amounts of soluble/free fluoride in the first SnF_2 dentifrices were limited further studies on alternative fluoride compounds were conducted and resulted in a shift to sodium fluoride based dentifrices. In 1987, data clearly demonstrated that there was a significant decline in caries, but still a high prevalence of gingivitis and gingival recession among adults [6]. Thus, the development of dentifrices provided not only a therapeutic effect against dental caries, but also against gingivitis came into focus. As SnF_2 combines both effects as well as being effective against sensitivity [7] and dental erosion [8], the focus has again been placed on this fluoride compound. Several new formulas stabilizing SnF_2 and increasing the amount of soluble/free fluoride have been investigated. The most frequently described compounds that have been included in the formulations are for example sodium gluconate [9], sodium fluoride [8], sodium hexametaphosphate [10] or amine fluoride [8]. Also the combination of sodium fluoride and stannous chloride has been used [11].

Although several studies analyzed the antierosive [11–13] and the antimicrobial [14] effects of the new SnF_2 containing formulas only two in vitro study analyzed the anticaries effect of the new formulas [15, 16]. In the first study, no significant differences in the change of mineral loss between a stannous chloride containing dentifrice and the positive control (NaF), but for both compared with the fluoride-free (fluoride negative) control, were observed after 5 days pH-cycling [15]. Contrastingly, in the second pH-cycling study stabilized stannous fluoride/sodium fluoride dentifrice containing sodium hexametaphosphate revealed a significantly lower gain in surface microhardness compared to solely sodium fluoride-containing and amine fluoride-containing dentifrices after 20 days [16]. The outcome used in the second study is best suited as a complementary measure to direct techniques (e.g., transversal microradiography) [17, 18], but it cannot be used on lesions that have a well-mineralized surface layer [19]. Thus, the results on stabilized stannous fluoride/sodium fluoride dentifrice containing sodium hexametaphosphate need to be further investigated, since it is known that fluoride of amine and sodium fluoride-containing dentifrices protects further demineralization and enhances remineralization by forming a hypermineralized surface layer [20, 21].

Several pH-cycling models have widely been used to analyze caries-preventive agents. Artificial enamel and/or dentine lesions are cycled between a demineralizing and remineralizing solution, mimicking oral pH-fluctuation. Depending on the severity and duration of the demineralization challenge inhibition of mineral loss (net demineralization) or increased mineral uptake (net remineralization) can be studied [22]. In both 'types' of pH-cycling models no antimicrobial effects can be analyzed and only the soluble/free (bioavailable) fluorides reacts with the substrate and can be released to the de- and remineralizing solutions [23]. Insoluble and inactive fluorides do not affect mineral loss. Since the new formulas have been developed to stabilize SnF_2 and to increase the amount of soluble/free fluoride, it is believed that these new formulations might show a similar remineralizing potential as sodium or amine fluoride-containing toothpastes. So far, this could only be shown for stannous chloride [15] but not for other SnF_2 containing formulas.

Thus, the aim of this in vitro study was to compare the caries-preventive effect of a stabilized stannous fluoride/sodium fluoride dentifrice containing sodium hexametaphosphate with those of a regular, solely sodium fluoride-containing and amine fluoride-containing dentifrice on pre-demineralized bovine enamel specimens using a pH-cycling model. We hypothesized that no significant differences in mineral loss would be observed between all dentifrices containing similar fluoride concentrations, but for all compared to a fluoride-free control or non-brushing.

Materials and methods

Specimen preparation

Bovine incisors were obtained from freshly slaughtered cattle (negative BSE test) and stored in 0.08 % thymol. Teeth were cleaned and 125 enamel blocks ($5 \times 3.5 \times 3$ mm) were prepared (Exakt 300; Exakt Apparatebau, Norderstedt, Germany). The enamel blocks were embedded in epoxy resin (Technovit 4071; Heraeus Kulzer, Hanau, Germany), ground flat and polished (4000 grit; silicon carbide, Phoenix Alpha, Wirtz-Buehler, Düsseldorf, Germany; Mikroschleifsystem Exakt, Exakt Apparatebau, Norderstedt, Germany) (Fig. 1).

Lesion formation

Specimens were partially covered with acid-resistant varnish (sound control). In each of 125 specimens two artificial lesions were created [pH 4.95; 5 days] (Fig. 1) [24]. The samples were transversally sectioned to the orientation of the protective acid-resistant varnish (Trennschleifsystem Exakt 300; Exakt Apparatebau) and polished (4000 grit; Mikroschleifsystem Exakt) until thin (100 μm) plano-parallel sections were obtained. Transversal microradiography (TMR) was used to determine baseline mineral loss (ΔZ_{B1} and ΔZ_{B2}) and lesion depth (LD_{B1} and LD_{B2}). Microradiographic assessment was performed only in the central part of the lesion, at 100 μm distance from the lesion margin. Eighty-five specimens with a mean (SD) baseline
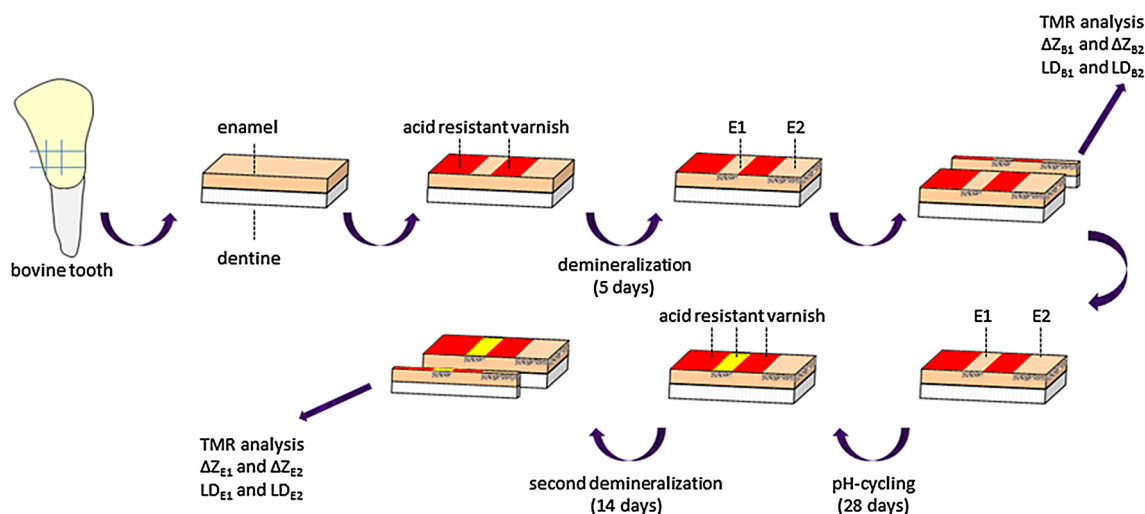
Fig. 1 Study design. Bovine enamel specimens with two artificial lesions each were prepared. *Baseline* mineral loss and lesion depth of both lesions were analyzed using transversal microradiography (TMR). After pH-cycling one lesion from each specimen was covered with acid-resistant varnish, while the remaining lesion was demineralized for another 14 days. Differences in integrated mineral loss ($\Delta\Delta Z$) and lesion depth (ΔLD) were calculated between values before and after pH-cycling ($\Delta\Delta Z_{E1}$ and ΔLD_{E1}) as well as before pH-cycling and after second demineralization ($\Delta\Delta Z_{E2}$ and ΔLD_{E2}) using TMR

mineral loss of 3393 (683) vol% × μm were selected and randomly allocated to five groups.

pH-cycling conditions

The pH-cycling lasted 28 days and conditions were chosen with a daily schedule of 2 cycles, where specimens were consecutively subjected to a demineralizing (0.3 h), a brushing and a remineralizing (11.6 h) phase. The remineralization solutions contained 1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4 and 20 mM Hepes, pH 7.0. The demineralization solution contained 0.6 μm methylhydroxydiphosphonate, 3 mM $CaCl_2$, 3 mM KH_2PO_4 and 50 mM acetic acid adjusted to pH 4.95 [24]. The pH-cycling solutions were refreshed with each cycle. Each specimen was cycled in 10-ml aliquots of the solutions. Thus, the amounts of each solution were large enough to prevent the solutions from becoming saturated with or depleted of mineral ions.

Surface treatment

After the demineralization periods each specimen was manually brushed by one of the authors (HZ) for 10 s (Oral-B Indicator; Proctor and Gamble, Schwalbach am Taunus, Germany) or not brushed and immersed in distilled water, as in group NB, the absolute negative control of the model. After brushing, the specimens remained immersed in the dentifrice slurries for further 10 s before the specimens were rinsed with distilled water for 30 s. Dentifrice slurries were prepared with deionized water in a ratio of 3:1 parts by weight and refreshed at the beginning of each experimental day. After pH-cycling one lesion from each specimen was covered with acid-resistant varnish, this area was denominated 'effect 1' (E1), while the remaining lesion was demineralized for another 14 days, representing the 'effect 2' (E2). The second demineralization was done with the same solution described previously [24]. The pH value was checked daily (pH-Meter GMH 3510; Greisinger, Regenstauf, Germany) and slight elevations were corrected with acetic acid or potassium hydroxide to maintain a constant pH between 4.98 and 5.02 during the demineralization period.

TMR

After the in vitro period thin plano-parallel sections were prepared again. Changes in mineral loss ($\Delta\Delta Z = \Delta Z_{Baseline} - \Delta Z_{Effect}$) and lesion depth ($\Delta LD = LD_{Baseline} - LD_{Effect}$) were calculated between values before and after pH-cycling for E1 ($\Delta\Delta Z_{E1}$ and ΔLD_{E1}) as well as before pH-cycling and after second demineralization for E2 ($\Delta\Delta Z_{E2}$ and ΔLD_{E2}) using transversal microradiographic images. Microradiographs of the enamel specimens were obtained and analyzed as described previously [25]. Furthermore, graphics of mean mineral density profiles were prepared for all groups with the TMR/WIM Calculation Program (v5.25; University of Groningen, the Netherlands).

Free fluoride analysis

Fluoride concentrations of all dentifrices were measured (Orion Autochemistry System 960; Fisher Scientific, Ulm,

Table 1 Study groups and composition of the dentifrices tested

Groups	AmF	NaF	SnF$_2$/NaF	FF (fluoride negative control)	NB (fluoride and brushing negative control)
Fluoride	(+)	(+)	(+)	(−)	(−)
Brushing	(+)	(+)	(+)	(+)	(−)
Fluoride compound[a]	AmF	NaF	SnF$_2$/NaF	None	None
Total fluoride [ppm F$^-$][a]	1400	1450	1100/350	(−)	(−)
Total stannous [ppm][a]	(−)	(−)	3417	(−)	(−)
pH of the dentifrice slurry[b]	4.8	6.1	5.9	8.1	(−)
Abrasives[b]	Hydrated silica	Hydrated silica	Hydrated silica	Hydrated silica, calcium carbonate	(−)
Active ingredient[a]	(−)	(−)	Sodium hexametaphosphate	(−)	(−)
Further ingredients[a]	Aqua, sorbitol/glycerin, hydroxyethylcellulose, aroma, limonene, CI 77891, sodium saccharin, hydrochloric acid	Aqua, glycerin, xanthan gum, sodium lauryl sulfate, aroma, limonene, sodium saccharin, zinc lactate, CI 77891	Glycerin, propylene glycol, PEG-6, aqua, zinc lactate, Ci 77891, sodium lauryl sulfate, sodium gluconate, aroma, chondrus crispus, trisodium phosphate, sodium saccharin, xanthan gum, silica	Aqua, sorbitol, xylitol, maris sal, CI 77891, xanthan gum, glycerin, disodium cocoyl glutamate, sodium cocoyl glutamate, Echinacea purpurea extract, Myrtus communis leaf water, propolis cera, alcohol, aroma, limonene, linalool, eugenol	(−)
Brand name[a]	Elmex Zahnpasta Kariesschutz (GABA, Germany)	Blend-a-med classic (P&G, Germany)	Blend-a-med pro-expert Zahnfleischschutz (P&G, Germany)	Lavera basis sensitiv Zahncreme classic (Weleda, Germany)	(−)

(+) present/done, (−) absent/not done

[a] Information provided by the manufacturers

[b] Information provided by this study

Germany) using a calibrated ion-specific electrode (type 96–09 BNC; Fisher Scientific) [26, 27]. For the measurements four fluoride solutions (3.8, 1.9, 0.38, and 0.19 mg/l) were prepared, since these concentrations are in the same range of the expected sample concentrations. The electrode potentials (mV) of the four standard solutions were measured and plotted against their concentrations (mg/l) in logarithmic scale. After calibration the fluoride concentration of the dentifrices were determined. For this, 200 mg of the dentifrices was diluted in 100 ml distilled water at room temperature. Four mL of each solution were centrifuged at 2500g and TISAB II (1:1; Fisher Scientific) was then added to control ionic strength. The percentage of free fluoride in relation to given total fluoride concentration (manufacture's information) was determined. Two solutions for each dentifrice were prepared and analyzed in triplicates.

Electron microprobe analysis

The content of stannous (Sn) being incorporated in the enamel surface of specimens in group SnF_2/NaF was measured using a JEOL Superprobe JXA 8900 electron microprobe at the Institute of Geosciences at Kiel University, equipped with five WD spectrometers. An accelerating voltage of 15 kV, a probe current of 15 nA and a probe diameter of about 5 μm were used. Characteristic X-rays were recorded using a TAP crystal for Na and Mg, a PETJ crystal for Cl and Ca and a PETH crystal for P and Sn. As standard materials natural tugtupite (Na, Cl), apatite (P), wollastonite (Ca), forsterite (Mg) and stannous oxide (Sn) were used. For both experimental setups (E1 and E2) three specimens of group SnF_2/NaF were prepared and analyzed in triplicates. The percentage of SnF_2 in relation to given total ions was determined. The calculated 1-sigma detection limit of Sn is about 200 ppm (0.02 wt%).

Statistical analysis

Data were analyzed using SPSS statistical software (SPSS 22.0; SPSS, Munich, Germany) and before analysis all variables were tested for normal distribution (Shapiro–Wilk test). Within one experimental group changes in mineral loss and lesion depth before and after pH-cycling (ΔZ_{B1} vs. ΔZ_{E1} and LD_{B1} vs. LD_{E1}) and after pH-cycling and second demineralization (ΔZ_{B2} vs. ΔZ_{E2} and LD_{B2} vs. LD_{E2}) were analyzed using t test. Analysis of variance (ANOVA) was used for pair-wise multiple-comparisons to detect differences in changes of mineral loss ($\Delta\Delta Z_{E1}$, $\Delta\Delta Z_{E2}$, ΔLD_{E1}, ΔLD_{E2}) between treatments. All tests were performed at a 5 % level of significance.

Results

After pre-demineralization specimens did not differ significantly in mineral loss between experimental groups ($p > 0.05$; Wilcoxon test). Mean (SD) baseline mineral loss was 3393 (683) vol% × μm. Due to preparation losses, final TMR analysis was performed with 13–15 specimens per group.

TMR—mineral density profiles

The mean mineral profiles of all groups for effects E1 and E2 can be seen in Fig. 2. After pH-cycling (E1) specimens of AmF and NaF revealed subsurface lesions without abrasive surface loss and specimens of SnF_2/NaF, FF and NC showed less mineralized surface layers than specimens of AmF and NaF. After second demineralization (E2), a (slightly) further increase in mineral loss could be observed for specimens of all groups (Table 2). Furthermore, for specimens of group SnF_2/NaF, FF and NB secondary lesion bodies beyond the original lesion fronts could be observed.

TMR—changes in mineral loss

After pH-cycling (E1) specimens of AmF, NaF and SnF_2/NaF remineralized and specimens of FF and NB demineralized as compared to baseline. However, significant changes in lesion mineral content between baseline and E1 (i.e., remineralization) were only observed for AmF and NaF ($p < 0.05$, t test). After second demineralization (E2) although only AmF and NaF remineralized, significant changes in lesion mineral content were observed for all groups ($p < 0.05$, t test) (Table 2). For comparisons between the groups NaF and AmF showed significantly different $\Delta\Delta Z_{E1}$ and $\Delta\Delta Z_{E2}$ values compared to NB and FF ($p < 0.05$; ANOVA test) (Table 2).

TMR—changes in lesion depth

After pH-cycling (E1) specimens of AmF, NaF, SnF_2/NaF and FF showed a decrease and specimens of NB showed an increase in lesion depth. However, significant changes in lesion depths between baseline and E1 measurements were only observed for NaF ($p < 0.05$, t test).

After second demineralization (E2) although only AmF and NaF showed a decrease in lesion depths, significant changes in lesion depths were observed for all groups except for AmF ($p < 0.05$, t test) (Table 2).

For comparisons between the groups no significant differences could be observed for ΔLD_{E1}. After second demineralization NaF and AmF showed significantly different ΔLD_{E2} values compared to NB and FF ($p < 0.05$;

Fig. 2 Mean mineral density profiles of the enamel specimens after pH-cycling (E1) and after pH-cycling plus 14 days demineralization (E2) were assessed. Specimens of AmF and NaF revealed subsurface lesions without abrasive loss after E1 and specimens of SnF₂/NaF, FF and NC showed lower mineralized surface layers than specimens of AmF and NaF. After E2 a (slightly) further increase in mineral loss could be observed for specimens of all groups. Furthermore, for specimens of group SnF₂/NaF, FF and NB secondary lesion bodies beyond the original lesion fronts could be observed
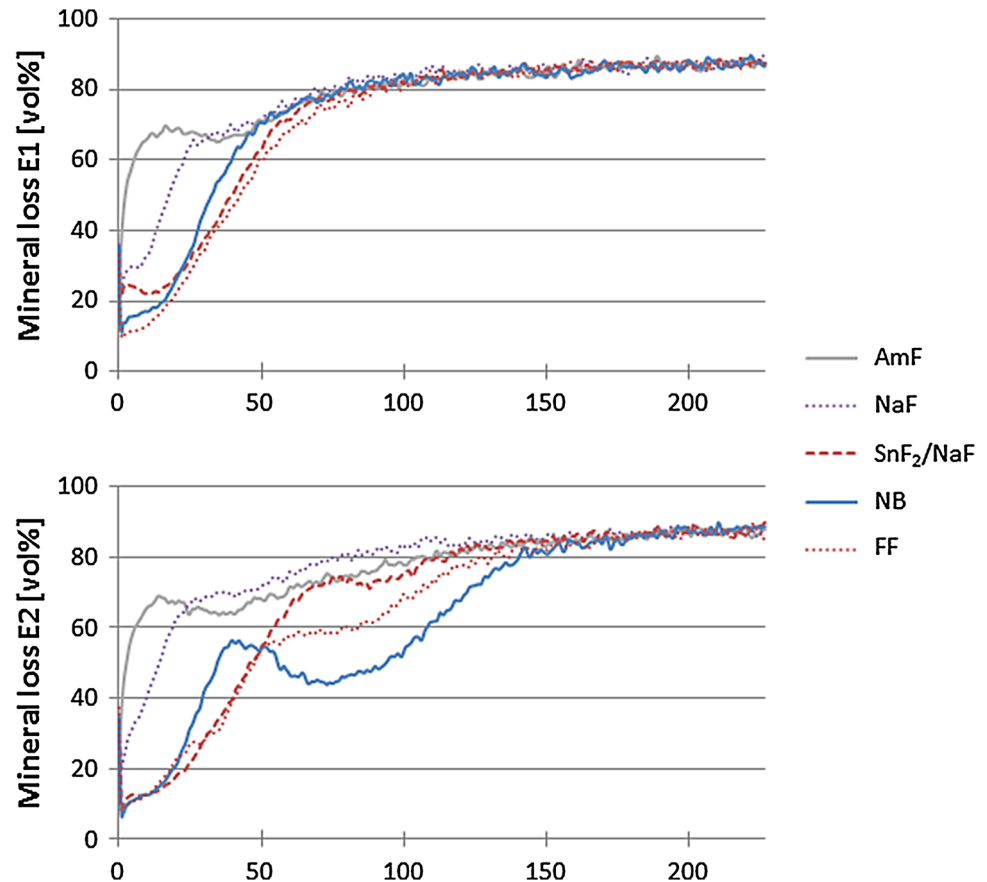
ANOVA test), whereas no significant difference for ΔLD_{E2} could be observed between SnF₂/NaF and NB or FF (Table 2).

Free fluoride and electron microprobe analysis

The percentages of free fluoride in relation to given total fluoride concentration (manufacture's information) (SD) were 89 (2.5) % for AmF, 85 (0.8) % for NaF and 86 (0.8) % for SnF₂/NaF. For the fluoride-free dentifrice no fluoride was measured. The maximum percentage of stannous in relation to total ions in the enamel surface layer was 0.27 wt% after pH-cycling and 0.25 wt% after second demineralization. No stannous could be detected at depths greater than 85 and 65 μm, respectively (Fig. 3).

Discussion

The present in vitro study compared the effect of a stabilized stannous fluoride/sodium fluoride dentifrice containing sodium hexametaphosphate with those of a regular solely sodium fluoride-containing and amine fluoride-containing dentifrice on the prevention of enamel caries

lesions progression in a pH-cycling model. Both dentifrices with either amine or sodium fluoride promoted remineralization significantly compared to fluoride-free dentifrice or non-brushing after pH-cycling as well as after a further demineralization. SnF₂/NaF dentifrice could not promote considerable remineralization in this biofilm-free pH-cycling model partially rejecting our hypothesis that also SnF₂/NaF containing dentifrice would significantly promote remineralization compared to fluoride-free dentifrice or non-brushing.

For specimens treated with AmF and NaF, a pronounced mineral gain was observed after pH-cycling and after second demineralization compared to SnF₂/NaF, FF and NB treatments—showing significantly higher changes in mineral loss than FF and NB. In order to interfere in the dynamics of dental caries formation different "reservoirs" of fluoride have been described [28]. However, the adsorbed fluoride is presumed to exhibit the main effect for caries prevention [28]. In the presence of free or soluble fluoride, hydroxyapatite will behave as fluorhydroxyapatite during future dissolution episodes as it shifts the critical pH for demineralization approximately 0.5–1.0 units to a more acidic critical pH [29]. The more fluoride is incorporated in the structure of the crystals, the higher the decrease in the

Table 2 Mean mineral loss and lesion depth as well as changes in mineral loss ($\Delta\Delta Z_{E1}$ and $\Delta\Delta Z_{E2}$) and lesion depth (ΔLD_{E1} and ΔLD_{E2}) for specimens before and after pH-cycling and after second demineralization

MTreatment	N	ΔZ_{B1} (vol% × μm)	ΔZ_{E1} (vol% × μm)	P_1	$\Delta\Delta Z_{E1}$ (vol% × μm)	P_2	ΔZ_{B2} (vol% × μm)	ΔZ_{E2} (vol% × μm)	P_1	$\Delta\Delta Z_{E2}$ (vol% × μm)	P_2
AmF	14	3339 (678)	1777 (374)	**<0.001**	1563 (767)	A	3426 (686)	2198 (684)	**<0.001**	1228 (871)	A
NaF	15	3354 (441)	2133 (1113)	**0.002**	1222 (1246)	AB	3240 (673)	2174 (919)	**0.005**	1065 (1235)	A
SnF$_2$/NaF	15	3599 (921)	3341 (823)	0.440	258 (1259)	BC	3632 (622)	4287 (615)	**0.001**	−654 (592)	B
FF	14	3662 (598)	3714 (1108)	0.876	−52 (1223)	C	3520 (500)	5029 (1239)	**0.001**	−1509 (1380)	BC
NB	13	2971 (535)	3123 (800)	0.526	−151 (835)	C	3297 (870)	5699 (1703)	**<0.001**	−2402 (1617)	C

LTreatment	N	LD_{B1} (μm)	LD_{E1} (μm)	P_1	ΔLD_{E1} (μm)	P_2	LD_{B2} (μm)	LD_{E2} (μm)	P_1	ΔLD_{E2} (μm)	P_2
AmF	14	88.7 (12)	79.5 (19)	0.137	9.2 (22)	A	91.2 (19)	89.4 (32)	0.823	1.8 (30)	AB
NaF	15	86.1 (28)	63.8 (24)	0.077	22.3 (45)	A	89.0 (21)	68.0 (31)	**0.048**	21.0 (38)	A
SnF$_2$/NaF	15	85.1 (21)	82.0 (22)	0.708	3.0 (30)	A	85.0 (15)	107.5 (17)	**<0.001**	−22.5 (15)	BC
FF	14	95.2 (19)	85.5 (20)	0.272	9.7 (32)	A	90.1 (13)	120.2 (28)	**0.004**	−30.1 (32)	C
NB	13	73.3 (12)	76.4 (14)	0.425	−3.1 (14)	A	77.7 (11)	130.6 (27)	**<0.001**	−52.9 (25)	C

Mean (SD) mineral loss and lesion depth for specimens before (B1/B2) and after the pH-cycling as well as pH-cycling plus demineralization (E1) and second demineralization (E2). Bold p_1 values indicate significant differences in mineral loss and lesion depth before and after the pH-cycling (E1) and second demineralization (E2). Different capital letters in the same column (P_2) indicate statistically significant differences between treatments ($p < 0.05$; ANOVA test)

critical pH of tooth mineral. Fluoride of amine and sodium fluoride-containing dentifrices could probably easily be incorporated in the structure presumably shifting the critical pH below the pH of our demineralization solution, hampering further mineral loss and an increase in lesion depth. Contrastingly, fluoride of stannous fluoride/sodium fluoride-containing dentifrice might not have been incorporated in the structure so easily since SnF$_2$ induces the formation of a relatively acid-resistant surface precipitate, limiting or delaying the direct contact of the acid with the mineral and presumably also the replacement of OH$^-$ by F$^-$ in hydroxyapatite [8, 13, 30]. The relatively acid-resistant surface precipitate seems to obstruct demineralization as well as remineralization. This is in agreement with our results showing almost no change in mineral loss and lesion depth in group SnF$_2$/NaF. Nonetheless, when simulating the progression of enamel caries lesions in the worst-case scenario (second demineralization) this surface layer did not seem to protect the enamel for further mineral loss when compared to treatments AmF and NaF. Fluoride of amine or sodium fluoride forming 'CaF$_2$-like' materials on the enamel surface protected the enamel surfaces at the first time and probably dissolved slowly over the time leading to a slight demineralization during 14 days of demineralization. Although the surface precipitate being formed by SnF$_2$ probably dissolved slowly over the time as well, the surface precipitate inhibited the incorporation of F$^-$ during remineralization before resulting in a smaller pool of fluoride that effectively protects the crystal from dissolution during the demineralization period. Thus, significantly more mineral loss for treatment SnF$_2$/NaF compared to AmF and NaF could be observed after second demineralization. Nonetheless, SnF$_2$/NaF showed a trend to hamper further demineralization compared to FF and NB.

Several studies demonstrated that acidic dentifrices have a greater capacity to enhance the penetration of mineral ions during remineralization compared with neutral dentifrices [31]. Thus, the deposition of mineral ions into the body of the lesion increases, resulting in a (significantly) reduced lesion depth [32]. In the present study the fluoride-free dentifrice (FF) revealed an alkaline pH, whereas the three fluoride-containing dentifrices (AmF, NaF SnF$_2$/NaF) revealed an acidic pH. The results of the AmF and NaF containing dentifrices were in agreement with previous studies [31, 32]; the more acidic dentifrice induced a higher remineralization than the less acidic one (while showing the same fluoride content). Interestingly, pH and fluoride (/ free fluoride) content of the SnF$_2$/NaF dentifrice were at the same level when compared to AmF and NaF; however, SnF$_2$/NaF could not promote remineralization considerably. Therefore, it might be speculated that the effect of the acidic pH of the dentifrice (enhancing mineral diffusion)

Fig. 3 The content of stannous (Sn) being incorporated in the enamel up to 85 μm depth of specimens in group SnF$_2$/NaF was measured using a JEOL Superprobe JXA 8900 electron microprobe. For both experimental setups (E1 and E2) three specimens of group SnF$_2$/NaF were prepared and analyzed in triplicates. The percentage (wt%) of stannous in relation to total ions present in enamel at each depth was determined
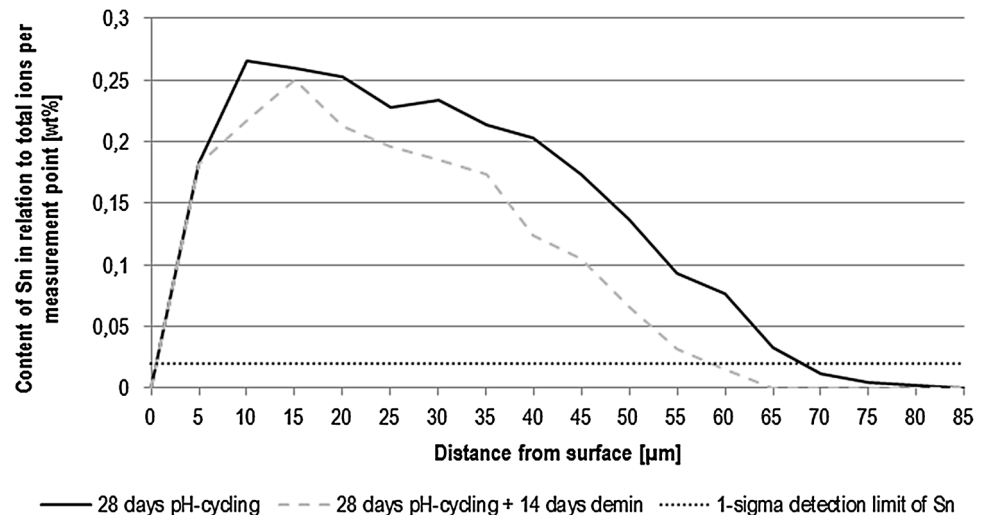
[31, 32] is overcome by the effect of the relative acid-resistant surface precipitate (hampering mineral diffusion) [8, 13, 30]. Furthermore, it might be speculated that the effect of increasing capacity to enhance remineralization with increasing acidity might be observed for certain fluoride compounds but not for all of them (e.g., SnF$_2$). However, this hypothesis cannot be answered with the result of the present study.

In the mean mineral density profiles, lower mineralized surface layers were detected for non-brushed specimens as well as for specimens brushed with FF and SnF$_2$/NaF. This might be interpreted as an indicator for abrasive losses during the pH-cycling period or the reduced presence of free fluoride at the sample–solution interface. In the present study, brushing was performed manually without an automatically controlled constant force and pace. Nonetheless, the author (HZ) was trained and calibrated prior to the pH-cycling period to brush all groups similarly. Therefore, abrasives losses would either be observed in all groups being brushed (which was not the case for AmF and NaF) or in none since abrasive ingredients of all dentifrices were similar. Furthermore, a lower mineralized surface layers could also be observed in specimens not being brushed at all. Thus, a complete abrasive surface loss in NB as well as in FF and SnF$_2$/NaF can be excluded.

By forming a mineralized surface layer, fluoride of amine and sodium fluoride-containing dentifrices protected further demineralization and enhanced remineralization [20, 21]. Contrastingly, mean mineral density profiles showed that in the other groups a mineralized surface layer could only be observed in a very limited extent. A less mineralized surface layer was not only observed in the absence of fluoride (FF and NB), but also in presence of SnF$_2$/NaF. Since fluoride incorporation in group SnF$_2$/NaF was probably inhibited (as discussed above) less

mineralization of the lesion was observed. Thus, not only FF and NB but also SnF$_2$/NaF showed a less mineralized surface layer than specimens of AmF and NaF.

The model used in this study mimics the dynamics of enamel caries formation. Demineralizing challenges were followed by remineralizing challenges. The experiment was a basic research study using a relatively mild demineralizing model being intended to result in slight net demineralization when no treatment (non-brushing) was carried out. The number of cycles in the protocol was different from other pH-cycling studies using up to 6 cycles [33, 34], but still allowed fluoride to act on the demineralizing as well as on the remineralizing process of dental caries. In the second part of our study the specimens were demineralized for 14 days to mimic the (fast) progression of enamel caries lesions in the worst-case scenario and to investigate whether one of the fluoride compounds showed protective effect against further demineralization. This might be an unrealistic sequence, since in the oral environment of most of the individuals demineralization is interrupted several times a day [29]. However, we demineralized the specimens for further 14 days to compare fluoride compounds when remineralization cannot keep up with demineralization.

Former in vitro [34] and in situ studies [35–39] showed that 'baseline substrate conditions' directly affects lesion response. What all authors concluded is that treatment groups should therefore be well-balanced with respect to baseline mineral loss and lesion depth. In the present study, specimens with a mean (SD) baseline mineral loss of 3393 (683) vol% × μm were selected to compare three dentifrices differing in fluoride compounds on a wide range of pre-demineralized bovine enamel. At first sight this seems to be in contrast to the conclusion of the former studies, since 'baseline substrate conditions' certainly also

influenced lesion response in the present study. Nonetheless, mean baseline mineral loss and lesion depth did not differ significantly between experimental groups. Thus, lesion response of the specimens within one experimental group probably differed, but between the experimental groups lesion responses were not affected differently.

Changes in mineral loss and lesion depth were calculated using TMR. With this technique mineral content of tooth material not being thicker than 100 μm can be quantified [40]. Using a polychromatic radiation as X-ray source an Al–Zn alloy step wedge can be used for mineral quantification since absorption spectra of both, tooth material and step wedge, are approximately similar [41]. Although the change in composition of the enamel and dentin does not affect the quantification, incorporation of polyvalent metal compounds may affect the quantification considerably [42]. Due to a higher absorption coefficient of stannous compared with hydroxyapatite [42], stannous incorporated in the enamel or deposited in the surface precipitate could result in a 'falsely' higher mineral content being interpreted as 'enhanced' remineralization. Previous studies demonstrated that a certain amount of stannous can be deposited as surface precipitates [30, 43] or incorporated in the enamel [43]. In the first study, investigating the effects of stannous (as $SnCl_2$ solution) on etched enamel, the amount of stannous deposited in the surface precipitate was up to 8.9 wt% directly after application and up to 8.2 wt% after 6 days of water storage [30]. In the second study, using solutions with different concentration of $SnCl_2$, 18.3 wt% stannous was detected as precipitate and 6 wt% incorporated into enamel [43]. The deposition as precipitates and the incorporation in the enamel structure were measured after cycling the specimens for 10 days. Contrastingly, in the present study the maximum percentage of stannous in relation to total ions present in enamel was much smaller, being of only 0.27 wt% after pH-cycling and 0.25 wt% after second demineralization. Even though, it is theoretically possible that even this very small amount of stannous could have influenced mineral quantification in the present investigation, it does not seem to have significantly changed our results. Actually if it would have influenced, this would be observable as enhanced mineralization for the SnF_2/NaF group, resulting from the higher X-ray absorption. This was not the case. In fact, even with a supposedly higher absorption, the SnF_2/NaF group showed less changes in mineral content (less remineralization in E1, or demineralization in E2) than both NaF and AmF groups and was not significantly different from FF. Thus indicating that the small amount of stannous incorporated to the enamel here did not notably influence the TMR results.

The anticariogenic effect of SnF_2 is based on two mechanisms. Firstly, SnF_2 delivers free or soluble fluoride to enhance remineralization as discussed above. Secondly, stannous ions, like other metals, exhibit an antibacterial and antiplaque effect. Stannous attaches to the bacterial surface, inhibits bacterial colonization, penetrates into the bacterial cytoplasm and interferes with the bacterial metabolism delaying bacterial growth [14, 44]. In this study a biofilm-free pH-cycling model was used. Thus, the antibacterial and antiplaque effect could not be analyzed. When analyzing both mechanisms simultaneously, it could previously be shown that stabilized stannous fluoride/sodium fluoride dentifrice containing sodium hexametaphosphate significantly reduces the 2-year caries increment relative to sodium fluoride [10]. It may, therefore, be assumed that in an in situ or in vivo study the tested SnF_2/NaF dentifrice led to an enhanced remineralization as seen for the other fluoride compounds.

In conclusion, both dentifrices with either amine or sodium fluoride promoted remineralization, whereas a stabilized stannous fluoride/sodium fluoride dentifrice containing sodium hexametaphosphate dentifrice did not promote remineralization in a biofilm-free pH-cycling model.

Compliance with ethical standards

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

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