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ORIGINAL ARTICLE

Influence of highly concentrated fluoride dentifrices on remineralization characteristics of enamel in vitro

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Abstract

Objectives The aim of this in vitro study was to evaluate the role of highly fluoridated dentifrice on remineralization characteristics of lowly and highly pre-demineralized enamel artificial caries lesions.

Methods Bovine enamel specimens were prepared (pH 4.95; 21 days) and discriminated in either lowly [L] or highly [H] pre-demineralized artificial caries lesions. Specimens with a mean $\Delta Z_{baseline,L}$ (95% CI) of 5120 (4995; 5245) vol.% × μm and a mean $\Delta Z_{baseline,H}$ of 8187 (8036; 8339) vol.% × μm were selected and randomly allocated to 12 groups ($n = 20$). Treatments during pH-cycling (28 days; 6 × 60 min demineralization/day) were brushing 2×/day with fluoride-free (0 ppm F^- [L_0/H_0]), 1100 ppm F^- [L_{1100}/H_{1100}], 2800 ppm F^- [L_{2800}/H_{2800}], 5000 ppm F^- [L_{5000}/H_{5000}], 5000 ppm F^- + glycerin [$L_{5000+glycerin}$/$H_{5000+glycerin}$], and 5000 ppm F^- + TCP [$L_{5000+TCP}$/$H_{5000+TCP}$] containing dentifrices. Dentifrice slurries were prepared with deionized water (1:3wt/wt). After cycling specimens presenting lesion surface loss were discarded and for the remaining 202 specimens, transversal microradiographic (TMR) analyses ($\Delta Z_{pH\text{-}cycle}$/$LD_{pH\text{-}cycle}$) were performed again. Changes in mineral loss ($\Delta\Delta Z = \Delta Z_{baseline} - \Delta Z_{pH\text{-}cycle}$) and lesion depth ($\Delta LD = LD_{baseline} - LD_{pH\text{-}cycle}$) were calculated.

Results Significant differences for ΔΔZ could be found between L_0, L_{1100}, and L_{5000} as well as H_0, H_{1100}, and H_{2800}/H_{5000} ($p \leq 0.01$; ANCOVA). Except for 0 ppm F^-, higher ΔΔZ could be found in highly compared with lowly demineralized specimens ($p \leq 0.004$; ANCOVA). After pH-cycling, a second lesion front could only be observed in H_{5000} and $H_{5000+TCP}$. The correlation between ΔΔZ and F^- was moderate for lowly and highly demineralized lesions ($r_L = 0.591$; $p_L < 0.001$; $r_H = 0.746$; $p_H < 0.001$), indicating a fluoride dose response for both.

Conclusion For both baseline substrate conditions, a dose response for fluoride could be revealed.

Clinical significance Remineralization characteristics of enamel directly depended on baseline mineral loss.

Keywords Enamel caries · Fluoride · Non-cavitated caries lesions · pH-cycling · Remineralization · Toothpastes

Introduction

The efficiency of dentifrices in order to inhibit caries lesion formation is supported by more than half a century of research [1, 2]. For coronal caries, a preventive effect of 23% could be found for dentifrices containing 1100–1250 ppm F^- compared with non-fluoride dentifrices [2]. For dentifrices containing 2400–2800 ppm F^-, the preventive effect increased to 36% [2]. Interestingly, in both reviews [1, 2], the preventive effect of dentifrices containing 5000 ppm F^- has not been analyzed. However, for dentifrices containing 5000 ppm F^-, it could recently be demonstrated that root caries incidence is significantly reduced and that significantly more root caries lesions are inactivated when compared to dentifrices containing 1100–1450 ppm F^- [3]. Furthermore, in vivo studies indicated a dose-response relationship between the preventive effect and fluoride concentration ([F^-]) [2, 4]. Thus, when analyzing fluoride dentifrices, in vitro or in situ respective models should be capable to demonstrate a (significant) fluoride dose response similar to the anticipated clinical response [4–7].

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Although several previous pH-cycling models have shown a dose response for fluoride concentrations in the range 0–1250 ppm F^- [8], only two models could demonstrate a fluoride dose response for fluoride concentrations up to 2800 ppm F^- [9, 10] or 5000 ppm F^- [11]. In the most recent study [9], three different methods of pre-demineralization were compared, and correlations between four different outcomes (lesion surface microhardness, mineral loss, lesion depth, and maximum mineral density of the lesion surface zone) were analyzed. The results of transversal microradiographic (TMR) data indicated a fluoride dose response for bovine and human enamel specimens. Under net-remineralizing conditions, the lowest mineral gain was observed for 0 ppm F^- whereas the highest mineral gain was observed for 2800 ppm F^-. However, no correlations with fluoride concentrations have been published. The second study revealed a fluoride dose response for dentifrices containing up to 3000 ppm F^- by comparing numerous different experiments with similar settings [10]. However, models slightly differed in study length, de- and remineralizing solutions, and the baseline mineral loss of the specimens. The third study analyzed dentifrices containing 0, 500, 1500, and 5000 ppm F^- [11]. When analyzing Ca^{2+} uptake and loss, a significantly increased remineralization and decreased demineralization could be observed in the first 3 days for 5000 ppm F^- compared with those for 1500 ppm F^-. However, when analyzing the mineral content by using TMR, no significant differences could be found between 5000 ppm F^- and 1500 ppm F^- (as NaF). In summary, all studies recommended that there is a need for further studies including lesions of various degrees of severity (baseline substrate conditions) before conclusions on optimal fluoride efficacy can be drawn.

In recent years, several different highly fluoridated dentifrices with different fluoride compounds (e.g., amine fluoride [AmF] instead of NaF [11]) or with additional active components (e.g., functionalized tricalcium phosphate (TCP)) have been introduced. Indeed, both studies indicated that 5000 ppm F^- as AmF or 5000 ppm F^- plus TCP, respectively, might promote remineralization significantly more effectively than 5000 ppm F^- as NaF.

Thus, the aim of the present study was, firstly, to reveal a dose response for fluoride dentifrices for lowly as well as highly demineralized enamel artificial caries lesions, secondly, to investigate the relation between baseline TMR data and re- and demineralization characteristics of enamel, and thirdly, to compare the caries preventive effect of different dentifrices containing 5000 ppm F^-. The null hypotheses were that, firstly, a significant correlation between mineral loss and fluoride concentration could be observed between lowly and highly demineralized lesions and that, secondly, no significant difference in the remineralizing effect of 5000 ppm F^- plus TCP and solely 5000 ppm F^- could be observed.

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 450 enamel blocks (5 mm × 3.5 mm × 3 mm) were prepared (Exakt 300; Exakt Apparatebau, Norderstedt, Germany) [12, 13]. 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).

Lesion formation

Two-thirds of the surface of each specimen was covered with nail varnish in order to assure enough mechanical and acid resistance for the untreated control area. To create artificial enamel caries lesions in uncovered areas, specimens were stored in a demineralization solution for 21 days (2.5 ml solution/mm^2 enamel surface) [14]. The solution contained 50 mM acetic acid, 3 mM $CaCl_2.H_2O$, 3 mM KH_2PO_4, 6 μM methylhydroxydiphosphonate, and traces of thymol (pH 4.95; 37 °C). During that period, pH was monitored daily and, if necessary, adjusted with small amounts of either 10% HCl or 10 M KOH to maintain a constant pH value. This way, 120 lowly demineralized lesions [S] with a mean (95% confidence interval [CI]) baseline mineral loss ($\Delta Z_{baseline,L}$) of 5120 (4995; 5245) vol.% × μm and a mean baseline lesion depth ($LD_{baseline,L}$) of 138 (134; 141) μm were chosen from the 450 specimens originally prepared. Furthermore, 120 highly demineralized lesions [A] with a mean (95% CI) baseline mineral loss ($\Delta Z_{baseline,H}$) of 8187 (8036; 8339) vol.% × μm and a mean baseline lesion depth ($LD_{baseline,H}$) of 185 (180; 189) μm were chosen.

pH-cycling condition

A computer-controlled pH-cycling and brushing machine [15] was used to simulate oral pH-fluctuation patterns and daily oral care. The pH-cycling (28 days) involved six demineralization periods of 1 h each (total 6 h/day) and six remineralization periods of at least 2 h during the day and a longer overnight period (total 18 h/day). The remineralizing solutions contained 1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, and 20 mM N-2-hydroxyethylpiperazine-N'2-ethanesulfonic acid (HEPES) as buffer at pH 7.0 (37 °C). 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 4.87 (37 °C) [14]. The pH-cycling solutions were refreshed with every cycle (6×/day). The amounts of each

solution were large enough to prevent the solutions from becoming saturated with or depleted of mineral ions (0.7 ml solution/mm^2 enamel surface).

Surface treatment and dentifrice slurries

The lowly and highly demineralized caries-like enamel lesions were randomly divided into 6 groups each, resulting in 12 subgroups. Before the first and last remineralizing phase of each day, the specimens were brushed for 10 s (Oral-B Indicator; Procter & Gamble, Schwalbach am Taunus, Germany) with the respective dentifrice slurry: fluoride-free [L_0/H_0], 1100 ppm F^- [L_{1100}/H_{1100}], 2800 ppm F^- [L_{2800}/H_{2800}], 5000 ppm F^- [L_{5000}/H_{5000}]), 5000 ppm F^- plus glycerin [$L_{5000+glycerin}$/$H_{5000+glycerin}$], and 5000 ppm F^- plus tricalcium phosphate [$L_{5000+TCP}$/$H_{5000+TCP}$] containing dentifrices (Table 1).

Dentifrice slurry remained on the specimens for another 110 s. Subsequently, specimens were perfused with distilled water to remove the slurry. In total, brushing procedure for each specimen lasted 120 s, hence simulating the recommended brushing and application time of 2 min [16]. The machine was adjusted to a constant brushing frequency of 60 strokes/min and a constant brushing load of 1.5 N [17].

Dentifrice slurries (except for $L_{5000+glycerin}$ and $H_{5000+glycerin}$) were prepared with deionized water in a ratio of 3:1 parts by weight and refreshed every 2 days. During this period, all slurries were stable. Only slurries of L_{5000} and H_{5000} dispersed into an aqueous and a dentifrice phase. To stabilize the emulsion of L_{5000} and H_{5000} glycerin, a humectant and emulsifier being found in several dentifrices were added. Thus, dentifrice slurries of $L_{5000+glycerin}$ and $H_{5000+glycerin}$ were prepared with deionized water (two parts), glycerin (one part), and dentifrice (one part) in a ratio 2:1:1.

Determination of free fluoride in the slurries

For the fluoride-containing dentifrices, total soluble fluoride concentrations in the slurries were determined as described previously [18].

Transversal microradiography analysis

After pH-cycling from each specimen, a slice of approximately 300 μm thickness (Exakt GmbH, Norderstedt, Germany) was obtained and subsequently ground flat and polished to a thickness of 100 μm (± 10 μm) using waterproof silicon carbide papers (FEPA grit sizes: 800, 1200, 2400, 4000; Struers). Parallelism of the specimens was tested with a digital micrometer with a precision of 0.001 mm (Mitutoyo, Japan). Contact microradiographs of the enamel specimens were obtained with a nickel-filtered copper (CuKa) X-ray source (PW 1730; Philips, Kassel, Germany) operating at 20 kV and 20 mA. The radiation source-to-film distance was 28 cm. The exposure time was 10 s, and a high-resolution film (Motion picture fine grain positive film 71337″; FUJIFILM Corporation Japan) was1 used and developed under standardized conditions according to the manufacturer's recommendations.

Microradiographs were digitalized by an image-analyzing system (Diskus software version 4.80; Königswinter, Germany) that is interfaced to a universal microscope (Leica DMRX; Germany) and a personal computer. A TMR software (version 5.25 by Joop de Vries, Groningen, Netherlands) was used to calculate the mineral loss ($\Delta Z_{baseline}$/$\Delta Z_{pH\text{-}cycle}$) and lesion depth ($LD_{baseline}$/$LD_{pH\text{-}cycle}$) before and after the pH-cycling [19]. 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) [20].

Calculation of integrated mineral loss and lesion depth

Mineral content was calculated by the TMR software based on the specimen's gray levels as described previously [12]. In short, the average mineral content of sound enamel was assumed to be 87 vol.% and the mineral density of sound enamel to be 2.88 g/cm^3 as measured by previous studies [21]. The lesion depth was calculated using a threshold of 95% of the mineral content of sound enamel (i.e., 82.7%). Thus, integrated mineral loss (ΔZ) and lesion depth (LD) could be calculated [22]. Changes in mineral loss ($\Delta\Delta Z = \Delta Z_{baseline} - \Delta Z_{pH\text{-}cycle}$) and lesion depth ($\Delta LD = LD_{baseline} - LD_{pH\text{-}cycle}$) were then calculated [20, 23].

Statistical analysis

Data were analyzed using SPSS statistical software (SPSS 22.0; SPSS, Munich, Germany). Variables were tested for normal distribution (Shapiro-Wilk test). Changes in mineral loss and lesion depth before and after pH-cycling ($\Delta Z_{baseline}$ vs. $\Delta Z_{pH\text{-}cycle}$ and $LD_{baseline}$ vs. $LD_{pH\text{-}cycle}$) were analyzed using two-tailed paired *t* test. Analysis of covariance (ANCOVA) for lowly and highly demineralized lesions was used to detect differences in the changes in mineral loss (ΔΔZ) and lesion depth (ΔLD) between interventions. More technically, the ANCOVA statistical model may be described as a general linear mixed model with TMR data (ΔZ, LD) and treatment as fixed effects. Correlation between [F^-] and ΔΔZ as well as between [F^-] and ΔLD were assessed using the Spearman's rank correlation coefficient. For this, only groups L_0, L_{1100}, L_{2800}, and L_{5000} and H_0, H_{1100}, H_{2800}, and H_{5000} were used. Spearman's rank correlation coefficient was

Table 1 Description of groups, toothpastes fluoride content and active ingredients

Group	Dentifrice	Fluoride content [ppm F^-]*	Active ingredient*	Free fluoride content (SD) [ppm F^-]#	Inactive ingredients*
$L_0/H_{0\ \text{Negative control}}$	Based on crest cavity protection[1], Procter & Gamble, Schwalbach am Taunus, Germany	0	–	12.3 (3.8)	Sorbitol, aqua, hydrated silica, sodium lauryl sulfate, trisodium phosphate, flavor, sodium phosphate, cellulose gum, carbomer, sodium saccharin, titanium dioxide, blue 1
L_{1100}/H_{1100}	Crest Cavity Protection, Procter & Gamble, Schwalbach am Taunus, Germany	1100	NaF	1281.6 (2.5) [116.5 (0.2) %]	Sorbitol, aqua, hydrated silica, sodium lauryl sulfate, trisodium phosphate, flavor, sodium phosphate, cellulose gum, carbomer, sodium saccharin, titanium dioxide, blue 1
L_{2800}/H_{2800}	Colgate® Duraphat® 2800 ppm fluoride toothpaste, Colgate-Palmolive Ltd., Guildford, UK	2800	NaF	2645.7 (122.3) [94.5 (4.4)]	Glycerol, purified water, sorbitol, silicas, macrogol 600, sodium lauryl sulfate, carmellose Sodium (E467), mint flavor, titanium dioxide, sodium saccharin
L_{5000}/H_{5000}	Colgate® Duraphat® 5000 ppm fluoride toothpaste, Colgate-Palmolive Ltd., Guildford, UK	5000	NaF	5066.6 (380.8) [101.3 (7.6)]	Liquid sorbitol (non-crystallizing), dental-type silica, dental-type silica (precipitated), macrogol 600, tetrapotassium pyrophosphate, xanthan gum, sodium benzoate (E211), sodium lauryl sulfate, spearmint flavoring (containing peppermint oil, carvone, spearmint oil, menthol, anethol, and lemon oil), saccharin sodium, brilliant blue FCF (E133) and purified water
$L_{5000+\text{glycerin}}/H_{5000+\text{glycerin}}$	Colgate® Duraphat® 5000 ppm fluoride toothpaste, Colgate-Palmolive Ltd., Guildford, UK	5000	NaF	5045.9 (511.9) [100.9 (10.2)]	Glycerol, liquid sorbitol (non-crystallizing), dental-type silica, dental type silica (precipitated), macrogol 600, tetrapotassium pyrophosphate, xanthan gum, sodium benzoate, sodium lauryl sulfate, spearmint flavoring (containing peppermint oil, carvone, spearmint oil, menthol, anethol, and lemon oil), saccharin sodium, brilliant blue FCF and purified water
$L_{5000+\text{TCP}}/H_{5000+\text{TCP}}$	Clinpro™ 5000 1.1%NaF anticavity toothpaste, 3M ESPE, St. Paul, USA	5000	NaF	4728.0 (69.0) [94.6 (1.4)]	Water, liquid sorbitol (non-crystallizing), glycerol, amorphous silica, polyethylene-polypropylene glycol, polyethylene glycol, flavorings, modified tricalcium phosphate, sodium lauryl sulfate, sodium carboxymethyl cellulose, saccharin sodium, titanium dioxide

* According to the manufacturer

According to the present measurements

[1] Manufactured by Procter & Gamble

also used to analyze correlation between baseline substrate conditions ($\Delta Z_{baseline}$) and change in mineral loss ($\Delta\Delta Z$). All tests were performed at a 5% level of significance.

Power calculations

The number of specimens per group was calculated on the basis of pre-studies (non-published data). The α-error was set at 5%. Considering the differences between the 0 and 1100 ppm fluoride dentifrice, the statistical power calculated for $\Delta\Delta Z$ was 85% (mean difference of 450 (SD 600)) and for ΔLD was 89% (mean difference of 4 (SD 5)). Dropout rate was assumed not to exceed 20%. Approximately 20 specimens should have been enrolled into the study for analyses of at least 16 specimens per groups. Since the retroperspective power analysis with 13 specimens has still provided a power of at least 88% for $\Delta\Delta Z_S$ (mean difference of 1026 (SD 801) and 82% for $\Delta\Delta Z_A$ (mean difference of 1423 (SD 1201), no additional specimens were included later.

Results

Mineral loss and lesion depth

After pre-demineralization, there was a significant difference in mineral loss ($p < 0.001$; ANCOVA) and lesion depth ($p < 0.001$; ANCOVA) between lowly and highly demineralized specimens. Furthermore, specimens within the subgroup of the respective baseline substrate condition (lowly/highly) did not differ significantly in mineral loss ($p = 1.000$; ANCOVA) and lesion depth ($p = 1.000$; ANCOVA) (Table 2). Mean (95% confidence interval) baseline mineral loss was 5120 (4995; 5245) vol.% × μm for lowly demineralized lesions and 8187 (8036; 8339) vol.% × μm for highly demineralized lesions. Due to losses during preparation, final TMR analysis was performed with 13–19 specimens per subgroup (Table 2). After pH-cycling, all subgroups showed signs of remineralization indicated by significantly lower ΔZ and LD values than before pH-cycling ($p \leq 0.012$; two-tailed paired t test), except for LD of L_0, H_0, and $L_{5000+TCP}$ ($p \geq 0.368$; two-tailed paired t test) (Table 2).

Highly demineralized lesions showed a significantly higher increase in mineral content ($\Delta\Delta Z$) ($p \leq 0.004$; ANCOVA) and lesion depth (ΔLD) ($p \leq 0.047$; ANCOVA) than the respective lowly demineralized lesions, expect for 0 ppm F^-. For both baseline substrate conditions, a significantly higher increase in mineral content ($\Delta\Delta Z$) could be observed for all fluoride groups (except for TCP-containing 5000 ppm F^-) compared with 0 ppm F^- ($p \leq 0.01$; ANCOVA, Fig. 1a, b). The application of 5000 ppm F^- induced a significantly higher gain in mineral content compared with that of 1100 ppm F^- ($p \leq 0.007$; ANCOVA). Furthermore, only for highly demineralized lesions, the application of 2800 ppm F^- induced a significantly higher gain in mineral content compared with that of 1100 ppm F^- ($p = 0.001$; ANCOVA).

Correlation analyses

According to the Spearman's rank correlation coefficient, a moderate and significant correlation could be found between F^- concentrations (0, 1100, 2800, 5000 ppm F^-) and $\Delta\Delta Z$ for lowly demineralized lesions ($r_{L,\Delta\Delta Z} = 0.591$; $p_{L,\Delta\Delta Z} < 0.001$) and a strong and significant correlation for highly demineralized lesions ($r_{H,\Delta\Delta Z} = 0.746$; $p_{H,\Delta\Delta Z} < 0.001$). The respective values for the correlations between ΔLD and F^- concentrations were $r_{L,\Delta LD} = 0.348$ and $p_{L,\Delta LD} = 0.005$ and $r_{H,\Delta LD} = 0.592$ and $p_{H,\Delta LD} < 0.001$.

Table 2 Mean (95% confidence interval) mineral losses and lesion depths after pre-demineralization ($\Delta Z_{baseline}$/$LD_{baseline}$) and after pH-cycling ($\Delta Z_{pH\text{-}cycle}$/$LD_{pH\text{-}cycle}$)

Intervention	N	$\Delta Z_{baseline}$ [vol.% × μm]		$\Delta Z_{pH\text{-}cycle}$ [vol.% × μm]		p^*	$LD_{baseline}$ [μm]		$LD_{pH\text{-}cycle}$ [μm]		p^*
L_0	18	4975	(4660; 5291)	3861	(3159; 4563)	*0.012*	127	(119; 135)	126	(110; 141)	0.647
L_{1100}	17	5204	(4875; 5533)	3136	(2611; 3660)	*< 0.001*	145	(1366; 154)	128	(113; 143)	*0.002*
L_{2800}	13	5152	(4820; 5484)	2352	(1678; 3025)	*< 0.001*	137	(126; 148)	116	(95; 136)	*0.008*
L_{5000}	17	5185	(4863; 5507)	2073	(1765; 2381)	*< 0.001*	142	(132; 153)	112	(99; 125)	*0.001*
$L_{5000+glycerin}$	18	5108	(4779;5436)	2620	(2272; 2968)	*< 0.001*	140	(130;150)	119	(105; 133)	*0.001*
$L_{5000+TCP}$	19	4839	(4216; 5463)	3334	(2777; 3891)	*< 0.001*	134	(124; 145)	128	(115; 141)	0.368
H_0	19	7628	(6719; 8537)	6158	(5564; 6752)	*< 0.001*	183	(171; 194)	186	(176; 197)	0.660
H_{1100}	14	8178	(7760;8596)	4539	(3706;5371)	*< 0.001*	181	(169; 194)	134	(107; 161)	*0.003*
H_{2800}	15	8272	(7906; 8638)	3598	(2927; 4270)	*< 0.001*	192	(177;206)	130	(102; 158)	*< 0.001*
H_{5000}	15	7851	(6909;8792)	3523	(3029; 4017)	*< 0.001*	182	(170; 194)	115	(94; 136)	*< 0.001*
$H_{5000+glycerin}$	19	8222	(7784; 8660)	4216	(3486;4946)	*< 0.001*	187	(175;198)	117	(96; 139)	*< 0.001*
$H_{5000+TCP}$	18	8156	(7749; 8563)	3920	(3394; 4446)	*< 0.001*	184	(172; 197)	135	(109; 162)	*< 0.001*

*Italicized p values indicate significant differences in mineral losses and lesion depths before and after pH-cycling (two-tailed paired t test)

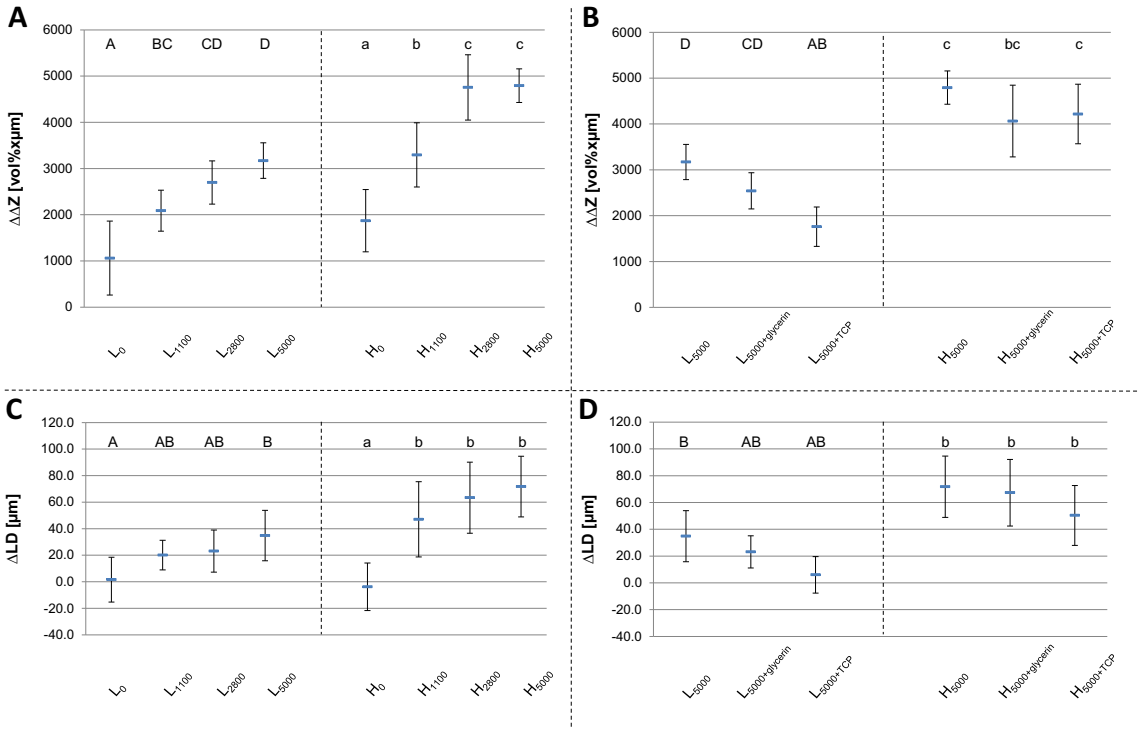

Fig. 1 Means with confidence intervals (95%) of the changes in mineral losses (ΔΔZ (**a**, **b**)) and lesion depths (ΔLD (**c**, **d**)). In each of the four diagrams, lowly demineralized (L) lesions can be seen at the left side and highly demineralized (H) ones on the right side. Different letters indicate significant differences between treatments among lowly (large caps) and highly demineralized (small caps) specimens ($p < 0.05$; ANCOVA)

Correlations between baseline substrate conditions ($\Delta Z_{baseline}$ and $LD_{baseline}$) and change in mineral loss (ΔΔZ) or change in lesion depth (ΔLD), respectively, are depicted in Table 3. With increasing fluoride concentrations, the significant correlation between $\Delta Z_{baseline}$ and ΔΔZ increased from moderate to very strong.

Mineral density of the lesion surface zone

All 202 specimens revealed subsurface lesions without abrasive surface losses (Fig. 2). During pH-cycling, the maximum (SZ_{max}) and minimum (SZ_{min}) mineral density of the lesion surface zone increased in all groups indicating the incorporation of minerals. After pH-cycling, a second layer of demineralized tissue could be observed in specimens of H_{5000}, $H_{5000+gylcerin}$, $L_{5000+TCP}$, and $H_{5000+TCP}$ (Fig. 2).

Table 3 Spearman's rank correlation coefficient and p values for the relation between baseline substrate condition ($\Delta Z_{baseline}$ and $LD_{baseline}$) and the changes in mineral loss (ΔΔZ) and in lesion depth (ΔLD) for the respective fluoride concentrations

Fluoride concentration (ppm F^-)	$\Delta Z_{baseline} \leftrightarrow \Delta\Delta Z$		$LD_{baseline} \leftrightarrow \Delta LD$	
	r value	p value*	r value	p value*
0	0.463	*0.004*	0.244	0.146
1100	0.476	*0.007*	0.100	0.579
2800	0.660	*< 0.001*	0.408	*0.031*
5000	0.817	*< 0.001*	0.607	*< 0.001*
5000 plus glycerin	0.650	*< 0.001*	0.610	*< 0.001*
5000 plus TCP	0.782	*< 0.001*	0.523	*< 0.001*

* Italicized p values indicate significant differences

Fluoride analysis

The free fluoride content (SD) and the percentage of free fluoride in relation to given fluoride content (SD) are given in Table 1. In fluoride-free dentifrice, a negligible amount of free fluoride was measured [12.3 (3.8) ppm F^-].

Discussion

The present in vitro study compared the caries preventive effect of dentifrices differing in fluoride concentration for lowly and highly demineralized caries-like enamel lesions. For both baseline substrate conditions, a significant correlation between mineral loss (ΔΔZ) and fluoride concentration in the dentifrices could be observed. However, both study null-hypotheses had to be rejected since the correlations varied between moderate for lowly and strong for highly demineralized specimens. Furthermore, for specimens treated with TCP-containing 5000 ppm F^-, a significantly lower mineral gain was observed compared with 5000 ppm F^-.

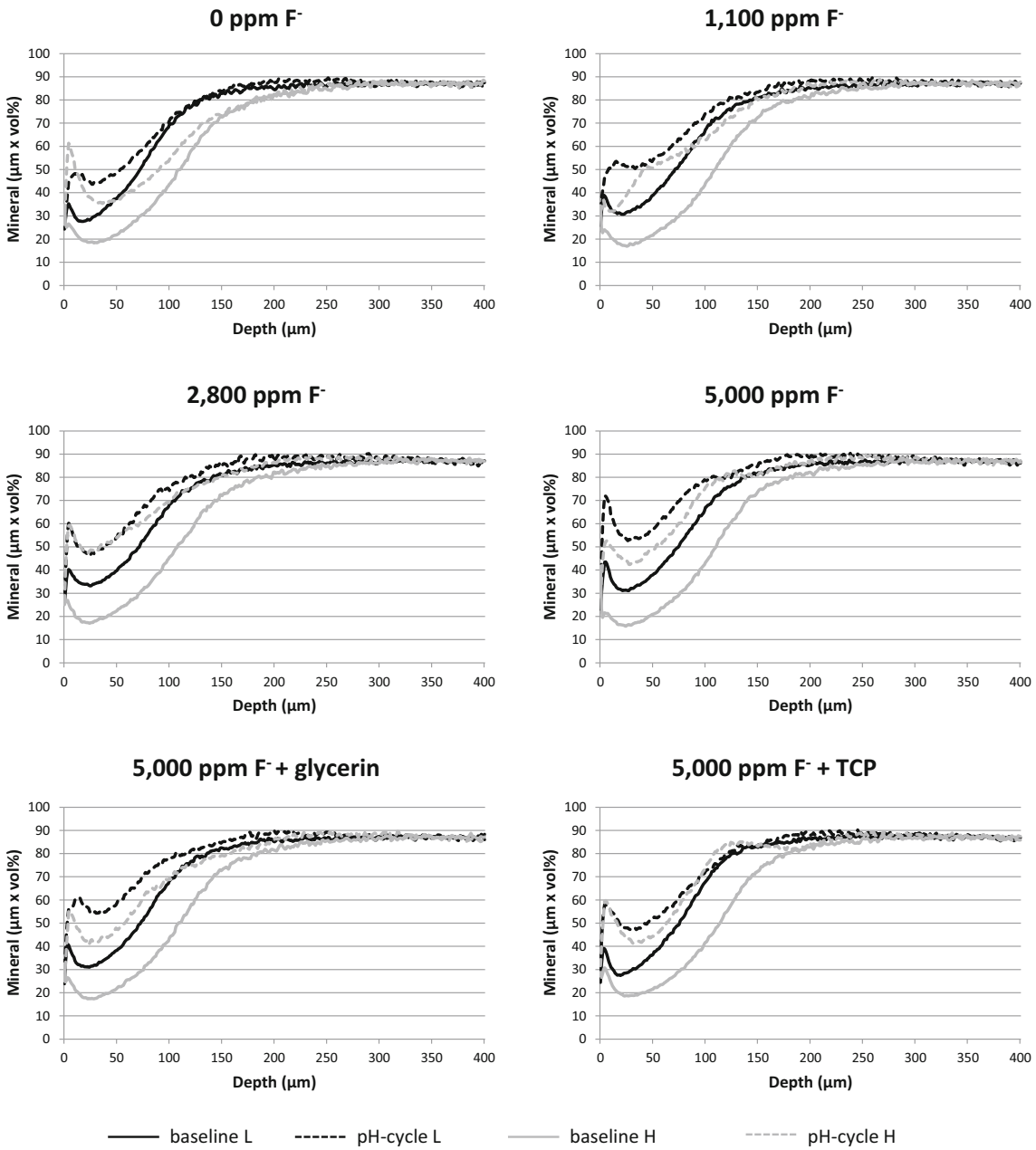

Fig. 2 Mean mineral density profiles of lowly (L) and highly demineralized (H) lesions were assessed after pre-demineralization (baseline) and after pH-cycling (pH-cycle) using the TMR/WIM calculation program. During pH-cycling, the maximum (SZ_{max}) and minimum (SZ_{min}) mineral density of the lesion surface zone increased in all groups indicating the incorporation of minerals

In the present remineralizing pH-cycling study, a fluoride dose response could be observed for highly as well as lowly demineralized lesions. For both substrate conditions, significant differences in ΔΔZ could be observed between 0, 1100, and 5000 ppm F^-. This was also reflected by the significant correlation between F^- concentration and ΔΔZ. On the one hand, this is in agreement with the re- and demineralization characteristics being observed in vivo [2, 24] and in situ [19, 20, 25]. On the other hand, this seems to be in contrast to a similar in vitro study [11]. In the previous study, no significant difference in the change of mineral loss between 5000 ppm F^- (as NaF) and 1500 ppm F^- (as NaF) was observed although a significantly increased remineralization and a decreased demineralization were observed between both groups when analyzing Ca^{2+} uptake and loss [11]. It might be speculated that the anti-caries effect of 1100 ppm F^- is (slightly) lower compared with the effect of 1500 ppm F^-, resulting in a slightly higher difference when compared with 5000 ppm F^-. Another possible reason for the different effect observed for the dentifrices in the previous study may be related to the baseline mineral loss, which was (presumably) not well-balanced. Due to the lower baseline mineral loss in group 5000 ppm

F^- compared with that in group 1500 ppm F^-, the potential for remineralization was lower for specimens of group 5000 ppm F^- compared with specimens of group 1500 ppm F^- [11]. Thus, that may have also contributed for the absence of significant difference between the two fluoride concentrations.

In previous in situ studies, an increasing potential for remineralization with increasing $\Delta Z_{baseline}$ and $LD_{baseline}$ under remineralizing conditions [25] and a decreasing potential for demineralization with increasing $\Delta Z_{baseline}$ and $LD_{baseline}$ under demineralizing conditions [20] has been observed. Indeed, under the present remineralizing conditions, highly demineralized lesions were significantly more prone to remineralization than lowly demineralized ones. This is also reflected in the significant correlations between $\Delta Z_{baseline}$ and $\Delta\Delta Z$; with increasing fluoride concentration, these correlations increased from moderate (0 ppm F^-) to very strong (5000 ppm F^-). Since the design of a pH-cycling study and the environment created by the model presumably have an overriding impact on its response (net demineralization or net remineralization) [8, 20, 25], it might be speculated that the dose-response effect observed for the various fluoride dentifrices in the present study might be the opposite under demineralizing conditions.

Specimens of H_{5000} and $H_{5000+TCP}$ presented a secondary lesion body (lamination) after pH-cycling. Lamination can be observed when fluorides are incorporated into the surface. The incorporation seems to induce larger and less soluble crystallites [26]. Furthermore, fluorohydroxyapatites buffer the solution to a lesser extent than hydroxyapatite [26]. Thus, when acids diffuse through the relatively large pores of the lesion, they are not neutralized [10, 27]. In consequence, the intact tissue beyond the original lesion is dissolved resulting in a second lesion body [10, 27]. In several previous pH-cycling studies, lamination characteristics varied widely. In one pH-cycling model, an inverse correlation between fluoride concentration and severity of the lamination was observed [10, 11]. Contrastingly, in other models, lamination was only observed for dentifrices containing 2800 ppm F^- [9] or 5000 ppm F^- (present data) but not for lower fluoride concentrations. Since different pre-demineralization agents were used in the mentioned studies, it might be speculated that the use of 8% methyl cellulose gel [10, 11], soluble carbopol [9], and soluble acetic acid (present study) resulted in pores with different sizes and different diffusion properties (for both fluoride and acids). In consequence, different lamination characteristics during pH-cycling were observed. This raises the question, which pre-demineralization protocol most closely mimics clinical conditions. In our view, this is still unclear.

In recent years, TCP as additional active component has been used to further increase the remineralizing effect of varnishes and dentifrices. TCP is supposed to act as Ca^{2+} and PO_4^{3-} reservoir, enhancing fluoride's efficacy without compromising the fluoride bioavailability [28]. During storage, the inactivation of the ions is prevented by carboxylic acid (fumaric acid), which is created in the manufacturing process of ball milling β-TCP [29]. If the fumaric acid gets into contact with an aqueous environment (e.g., saliva), the β-TCP–fumaric acid interface breaks. In consequence, Ca^{2+} and PO_4^{3-} are released [30]. For varnishes containing NaF plus TCP, the remineralizing effect for enamel (and dentin) varied between significantly inferior [31], as good as [30, 32] and significantly superior [33] when compared with NaF (without TCP). Regarding the anti-caries effect of dentifrices containing 5000 ppm F^- plus TCP, only surrogate outcomes as photomicrographs [34] have been used in vitro. However, in situ, a significantly higher mineral gain could be observed for dentifrices containing 5000 ppm F^- plus TCP when compared with dentifrices containing solely 5000 ppm F^- [35]. In contrast to our hypothesis, a significantly lower mineral gain was observed for $L_{5000+TCP}$ when compared with that for L_{5000}. Interestingly, in the previous and in the present study, different pre-demineralization agents were used. Thus, it might be speculated that the use of an acidified gel system [35] and soluble acetic acid (present study) resulted in pores with different sizes and different diffusion properties (as discussed above). In consequence, different de- and remineralization characteristics during the in vitro and the in situ period might be observed.

Although the present model revealed a dose-response characteristic for fluoride dentifrice, dentifrices not only differed in fluoride (and TCP) content but also in other inactive ingredients. It might, thus, be speculated that the observed dose-response characteristic for fluoride was influenced by other ingredients (e.g., antimicrobial or abrasive ingredients). However, all 202 specimens revealed subsurface lesions without abrasive surface losses, and no antimicrobial interferences are expected in a chemical caries model. Nonetheless, it would be interesting to test dentifrices differing only in their fluoride (and TCP) content.

The model used in this study mimics the dynamics of enamel caries formation. However, pH-cycling models have several limitations [8]: (1) Demineralizing challenges were followed by remineralizing challenges. These changes were much faster than those expected to occur in in vivo conditions; (2) The brushing procedure did not adequately simulate topical use and clearance of products from the oral cavity; (3) The complex intraoral condition formed by the bacterial biofilm, saliva, and eating behavior could not be simulated; (4) The surface area/solution ratio and saliva/plaque fluid composition being found in vivo were not simulated. Therefore, the present findings should further be investigated in clinical studies.

Within the limitation of an in vitro study, it can be concluded that the present pH-cycling model was capable to reveal a dose-response characteristic for fluoride dentifrice to increase further remineralization for different baseline substrate conditions. Moreover, it could be shown that even under in vitro conditions, remineralization characteristics directly depend on

specimens' baseline substrate conditions. Furthermore, the dentifrice containing 5000 ppm F^- revealed the highest anti-caries effect, whereas the dentifrices containing 5000 ppm F^- plus TCP did not promote remineralization significantly more than dentifrices containing 1100 ppm F^-.

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Compliance with ethical standards

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Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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