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ORIGINAL ARTICLE

Effect of NaF, AmF, KF gels and NaF toothpaste combined with a saliva substitute on dentin lesions in vitro

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Abstract

Objective The aim of the present in vitro study was to evaluate the remineralizing effects of NaF, AmF, KF gels and NaF toothpaste in combination with a potentially demineralizing saliva substitute (Glandosane; pH = 5.1) being widely used in Germany.

Methods In each of 120 dentin specimens, three artificial lesions were created. One lesion was covered for analysis of pre-demineralization (ΔZ_B). Treatments during pH cycling (3 × 1 h demineralization/day [pH = 5.0] and 3 × 3 h Glandosane/day; 12 h 100%humidity) were as follows: no treatment (NT), application (5 min,2×/day) of 12.500 ppm F^- [pH = 6.04] (NaF-gel$_1$), 12.500 ppm F^- [pH = 7.34] (NaF-gel$_2$), 12.500 ppm F^- [pH = 5.82] (AmF-gel), 1450 ppm F^- [pH = 7.35] (KF-gel), and 5000 ppm F^- [pH = 8.14]; (NaF-TP) for 7 days (E1). Subsequently, from each specimen, one lesion was covered, while the remaining lesion was cycled for another 7 days (E2). Differences in integrated mineral loss ($\Delta\Delta Z_{E1}/\Delta\Delta Z_{E2}$) were calculated between values before and after pH cycling.

Results Mean (95%CI) ΔZ_B was 3851 (3762;3939) vol% × μm. Except for NaF-gel$_2$ and NaF-TP, specimens of all other groups further demineralized. Only NaF-gel$_2$ induced a significant gain in mineral content ($p \leq 0.004$; paired t test). Significant differences in the change of mineral loss were found between NT and all fluoride groups for both $\Delta\Delta Z_{E1}$ and for $\Delta\Delta Z_{E2}$ ($p < 0.05$, Bonferroni post hoc test). However, only NaF-gel$_2$ and NaF-TP induced remineralization.

Conclusion Under the in vitro conditions chosen, all fluoride agents could significantly hamper the adverse effects of a demineralizing saliva substitute.

Clinical significance In combination with a demineralizing saliva substitute, slight mineral gain was only observed for neutral NaF-gel$_2$ and 5000 ppm F^- toothpaste.

Keywords Remineralization · Fluorides · Saliva · Dentin · Non-cavitated caries lesions · pH cycling · Hyposalivation

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Introduction

Xerostomia is the sequel of several diseases and therapies, as for example head and neck radiotherapy [1] or Sjögren's syndrome [2]. A decreased salivary flow leads to a reduced remineralizing potential of saliva resulting in reduced buffer capacity within cariogenic dental biofilms [3]. Therefore, patients suffering from xerostomia not only lack oral comfort but are also often afflicted with rampant caries—in particular in dentin [4–6].

Saliva substitutes are supposed to relieve the sensation of dry mouth [7] and focus on preventing lesion progression through improving patient's oral hygiene. A number of artificial salivas with different chemical compositions demonstrated neutral or remineralizing effects on dental hard tissues [8].

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One particular saliva substitute—being widely used in hospitals and care facilities in Germany—is Glandosane (Cell Pharm, Hannover, Germany) [9]. Glandosane is a carboxymethylcellulose (CMC)-based saliva substitute which is well-accepted by the patients because of pricing, taste, and handling [10]. However, in vitro, it has been observed that Glandosane induces demineralizing effects on enamel and dentin [8, 11, 12].

A systematic Cochrane review suggests that the application of highly concentrated fluoride gels results in a caries-inhibiting effect in healthy children and adolescents [13]. Moreover, for irradiated patients, highly fluoridated products such as gels are commonly recommended for caries prevention [14], since the topical use of these agents might trigger increased remineralization of enamel and dentin. Previous in vitro studies indicated that the additional use of fluoride agents in combination with a potentially demineralizing saliva substitute reduces mineral loss when compared with the saliva substitute alone [15]. The demineralizing effects of Glandosane were reduced by a mouthrinse containing AmF-SnF_2 (250 ppm F^-), a mouthrinse containing AmF-KF (250 ppm F^-), a gel containing NaF (12,500 ppm F^-), and a gel containing AmF (12,500 ppm F^-) [15]. Furthermore, in another study, the application of a NaF-gel (12,500 ppm F^-) and a highly fluoridated NaF toothpaste (5000 ppm F^-) could significantly hamper the demineralizing effect of Glandosane [16]. Interestingly, brushing with the highly fluoridated toothpaste seems to result not only in a reminalization being several time higher compared to no brushing [11, 12, 17], the application of AmF-KF mouthrinse [11, 17], or brushing with AmF toothpaste [11, 12, 17] but also compared to the application of NaF-gel [16]. However, in the previous in vitro studies, specimens were solely stored in remineralizing solutions for either 5 or 10 weeks, respectively. No demineralization solution was used intermittently to simulate oral pH fluctuations that occur in the oral environment frequently.

Thus, the purpose of the present in vitro study was to compare NaF, AmF, KF gels and NaF toothpaste in combination with a potentially demineralizing saliva substitute in a net-demineralizing pH cycling model. We hypothesized that no significant differences in mineral loss would be observed between the fluoride agents but for all compared with the no treatment control.

Material and methods

Specimen preparation

Two hundred dentin specimens ($6 \times 4 \times 4$ mm^3) were prepared from 50 extracted bovine incisors (negative BSE test) and stored in aqueous 0.08% thymol solution. Subsequently, all specimens were embedded in epoxy resin (Technovit 4071; Heraeus Kulzer, Wehrheim, Germany), and dentin surfaces were ground flat and hand-polished (waterproof silicon carbide papers, FEPA grit sizes: 1200 and 4000; Struers).

Lesion formation

Half of the specimens were covered with acid-resistant nail varnish (Jet Set; L'oréal, Paris, France) (sound control windows; SC) (Fig. 1). Three artificial lesions were created by immersion in a solution of constant composition containing 47.6 μM NaF, 2.2 mM $CaCl_2 \times 2H_2O$, 2.2 mM KH_2PO_4, 50 mM CH_3COOH, and 10 mM KOH (Merck, Darmstadt, Germany) at pH 5.0 in an incubator (37 °C; BR 6000; Heraeus Kulzer) for 5 days [16] (demineralized treatment areas, windows DT_B, DT_{E1}, DT_{E2}). The pH was monitored daily with a pH meter (GMH 3510; Greisinger, Regenstauf, Germany), and slight changes were either corrected with potassium hydroxide (1 M; Merck) or with hydrochloric acid (0.1 M; Merck). After 5 days of demineralization, six randomly chosen specimens were evaluated to control for similar and homogenous demineralization (± 150-μm depth) using transversal microradiography (TMR). Subsequently, one of these lesions (DT_B) was covered with acid-resistant nail varnish for analysis of pre-demineralization (ΔZ_B).

pH cycling condition

Specimens were randomly allocated to six groups ($n = 20$) and pH cycled for 7 days (E1). Subsequently, from each specimen, one lesion was covered, while the remaining lesion was cycled for another 7 days (E2) (Fig. 1). Conditions were chosen with a daily schedule of 3 cycles where specimens were consecutively subjected to a demineralizing (1 h) and a potentially demineralizing (3 h) "saliva substitute" phase (Glandonsane; Cell Pharm, Hannover, Germany). In-between the cycles, specimens were rinsed with water (10 s). Overnight, all specimens were stored (12 h) in 100% humidity mimicking a neutral phase during bed time. The demineralizing solution contained 47.6 μM NaF, 2.2 mM $CaCl_2 \times 2H_2O$, 2.2 mM KH_2PO_4, 50 mM CH_3COOH, and 10 mM KOH (Merck, Darmstadt, Germany). The pH cycling solutions were refreshed every second day.

Surface treatment

Before the first and last saliva substitute phase of each day, the respective fluoride agents were applied without any force using either a toothpick (gel) or were brushed with the toothpaste slurry for 5 min: no treatment [NT], 12,500 ppm F^- [AmF-gel], 12,500 ppm F^- [NaF-gel_1], 12,500 ppm F^- [NaF-gel_2], 5000 ppm F^- [NaF-TP], and 1450 ppm F^- [KF-gel] (Table 1). Gel treatments were not dissolved in water. Slurries were freshly prepared at each experimental day (one part toothpaste to three parts bi-distilled water, by weight).

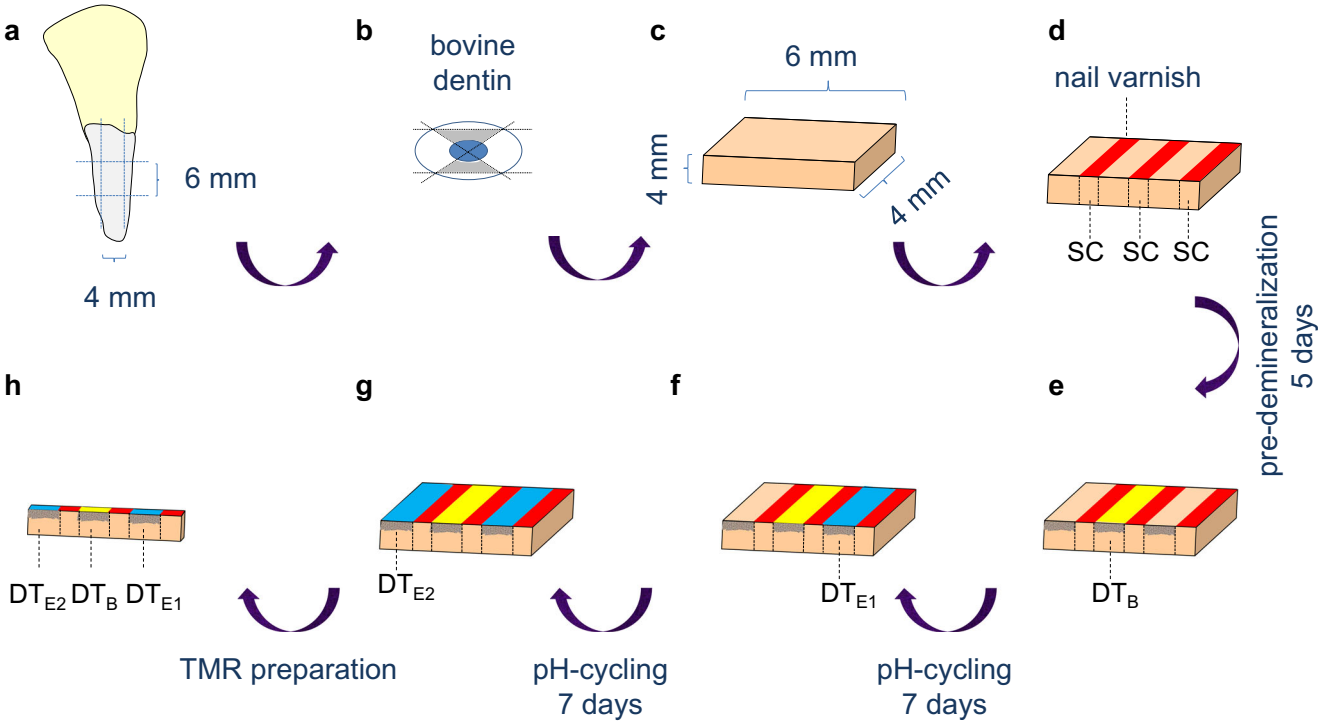

Fig. 1 Specimen preparation. **a** Frontal view of bovine front tooth. **b** Transversal section of bovine root. **c** Obtained specimens (6 mm × 4 mm × 4 mm). **d** Specimen covered with acid-resistant nail varnish (sound control windows SC). **e** pre-demineralized specimen (yellow, demineralized treatment area, windows DT_B). **f**, **g** Control area and treatment area (blue, demineralized treatment areas, windows DT_{E1} and DT_{E2}). **h** Obtainment of the 100-μm slices for baseline TMR analysis

Transversal microradiography

After pH cycling, thin sections (100 ± 10 μm) from all specimens were prepared using waterproof silicon carbide papers (SiC, grit sizes 1200 + 4000; Buehler, Düsseldorf, Germany) [18]. Exact section size was reevaluated with a digital micrometer (precision of 0.001 mm; Mitutoyo, Japan), and contact microradiographs of the dentin specimens were obtained (PW 1730/10; Philips, Eindhoven, Netherlands; 20 kV, 10 mA). The radiation source-to-film distance was 34 cm, with a 10-s exposure time, and a high resolution film (Motion picture fine grain positive film71337″; FUJIFILM, Japan) was used and developed under standardized conditions according to the manufacturer's recommendations. Microradiographs were studied with a digital image-analyzing system (CCD video camera Modul XC77E; Sony, Japan) that was interfaced to a microscope (Axioplan; Zeiss, Oberkochen, Germany) and a computer [19]. Furthermore, graphics of mean mineral density profiles were prepared for all groups with the TMR/T-WIM Calculation Program (Version 2.0.27.2, Inspector Research System BV, Amsterdam, Niederlande) [20, 21].

Calculation of integrated mineral loss and lesion depth

The difference between the mineral content (vol%) in sound control and demineralized dentin over the total dimension of the lesion was calculated using TMR software. Differences in integrated mineral loss ($\Delta\Delta Z_{E1} = \Delta Z_B - \Delta Z_{E1}$ / $\Delta\Delta Z_{E2} = \Delta Z_B - \Delta Z_{E2}$) and lesion depth ($\Delta LD_{E1} = LD_B - LD_{E1}$ / $\Delta LD_{E2} = LD_B - LD_{E1}$) between values before and after pH cycling were calculated [22].

Statistical analysis

Data were analyzed with SPSS statistical software (SPSS 25.0; SPSS, Munich, Germany). Variables were tested for normal distribution (Shapiro-Wilk test). Changes in mineral loss and lesion depth before and after pH cycling were analyzed using two-tailed paired *t* tests [23]. One-way ANOVA and Bonferroni post hoc tests were used to detect differences in changes of mineral loss ($\Delta\Delta Z_{E1}$ / $\Delta\Delta Z_{E2}$) and lesion depth (ΔLD_{E1} / ΔLD_{E2}) between the treatment groups. All tests were performed at a 5% level of significance.

Power calculation

The number of specimens per group was calculated based on previous studies (non-published data). The α-error was set at 5%. Considering the differences between NT and NaF_1 [$\Delta\Delta Z_{E1}$: mean difference of 800 (SD 500); $\Delta\Delta Z_{E2}$: mean difference of 2000 (SD 700)], the statistical power calculated for $\Delta\Delta Z_{E1}$ was > 80% and for $\Delta\Delta Z_{E2}$ > 80%. Dropout rate was

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

Group	Name	Toothpaste/gel	Fluoride content (ppm F^-)	Active ingredient	pH	Inactive ingredients*
NT Negative control	NT		0	–	–	–
AmF-Gel	Elmex Gelée	CP GABA GmbH, Hamburg, Germany	12.5	AmF (olafluor, dectafluor)	4.82	Purified water, propylene glycol, hydroxyethyl cellulose, saacharin, aroma
NaF-Gel$_1$	ProSchmelz Fluorid Gelée	GlaxoSmithKline Consumer Healthcare GmbH & Co. KG, München, Germany	12.5	NaF	6.04	Purified water, disodium, carbomer 956, sodium dodecyl sulfate, sodium saccharine, aroma, sodium hydroxide, patent blue V
NaF-Gel$_2$	Paro Fluor Gelée	Paro® Esro AG, Kilchberg, Switzerland	12.5	NaF	7.34	Aroma, lauryl sulfate, sodium saccharine, patent blue, Methyl, propylparaben sodium
KF-Gel	Fluor Protector Gel	Ivoclar Vivadent AG, Schaan, Principality of Liechtenstein	1.45	KF	7.65	Purified water, xylitol, hydroxyethyl cellulose, ethanol, calcium glycerophosphate, laureth-23, panthenol, methylparaben, aroma, sodium saccharin
NaF-TP	Duraphat 5000 ppm Fluoride Toothpaste	CP GABA GmbH, Hamburg, Germany	5	NaF	8.15	Liquid sorbitol (non-crystallizing; 70%), silica, precipitated silica, macrogol 600, potassium diphosphate, xanthan gum, sodium benzoate, sodium, aroma, sodium saccharin, brilliant blue FCF, purified water

assumed not to exceed 20% [24]. Approximately 20 specimens should have been enrolled into the study for analyses of at least 16 specimens per group. Since the retrospective power analysis with 14 specimens still provided a power of > 80% for $\Delta\Delta Z_{E1}$ (mean difference of 677 (SD 533)) and > 80% for $\Delta\Delta Z_{E2}$ (mean difference of 2341 (SD 785)), no additional specimens were included in the study.

Results

Mineral loss and lesion depth

For baseline mineral loss (ΔZ_B) and lesion depth (LD_B), no significant difference between the groups could be observed ($p > 0.05$, ANOVA, Table 2). Mean (95%CI) ΔZ_B was 3851 (3762; 3939) vol% × μm, and LD_B was 188 (182; 194) μm). Due to losses during preparation, TMR analysis was performed with 14–19 specimens per subgroup (Table 2).

After pH cycling specimens of NT, NaF-gel$_1$, AmF-gel, and KF-gel showed signs of demineralization indicated by significantly higher ΔZ and LD values than before pH cycling ($p \leq 0.037$, paired t test) except for LD_{E1} of AmF-gel. Contrastingly, specimens of NaF-gel$_2$ and NaF-TP showed signs of remineralization indicated by lower ΔZ values. However, only NaF-gel$_2$ induced a significant gain in mineral content after 7 and 14 days ($p \leq 0.025$, t test, Table 2).

Significant differences in the change of mineral loss ($\Delta\Delta Z$) and lesion depth (ΔLD) were found between NT and all fluoride groups after 7 as well as after 14 days ($p \leq 0.006$, Bonferroni post hoc test). NaF-gel$_2$ showed significantly higher values for $\Delta\Delta Z_{E1}$ and $\Delta\Delta Z_{E2}$ compared to NT, NaF-gel$_1$, AmF-gel, and KF-gel. Furthermore, NaF-TP presented significantly higher values for $\Delta\Delta Z_{E1}$ compared to NT, NaF-gel$_1$, AmF-gel, and KF-gel (Fig. 2).

Mineral density of the lesion surface zone

The respective mineral distribution profiles of the lesions before and after pH cycling are shown in Fig. 3. After pH cycling, a second layer of demineralized tissue (lamination) could be observed in specimens treated in particular with KF-gel but also for NaF-TP.

Discussion

The present in vitro study evaluated the remineralizing effects of different highly concentrated fluoride agents in combination with a potentially demineralizing saliva substitute. Compared with the negative control, all fluoride agents could significantly hamper further demineralization. However, NaF-gel$_2$ was significantly superior to the NaF-gel$_1$, AmF-gel, and KF-gel. Furthermore, NaF-gel$_2$ was the only agent inducing a slight remineralization of the dentin specimens. For this reason, our hypothesis was partially rejected.

Glandosane is supposed to relieve the sensation of dry mouth of patients suffering from xerostomia [10]. However,

Table 2 Mean (95% confidence interval) mineral losses and lesion depths for specimens before and after pH cycling

Intervention	*n*	ΔZ_B (vol% × µm)			ΔZ_{E1} (vol% × µm)			pe1	ΔZ_{E2} (vol% × µm)			pe2
NT	19	3895	(3684;4105)	A	5507	(5197;5816)	A	*< 0.001*	6562	(6174;6950)	A	*< 0.001*
NaF-Gel$_1$	15	3946	(3644;4249)	A	4882	(4484;5279)	AB	*< 0.001*	4272	(3979;4565)	B	0.130
AmF-Gel	17	3761	(3550;3973)	A	4284	(3901;4666)	BC	*0.004*	4222	(4022;4422)	B	*0.002*
NaF-Gel$_2$	15	3965	(3742;4188)	A	3639	(3256;4021)	C	*0.025*	3385	(3091;3678)	C	*< 0.001*
KF-Gel	17	3975	(3794;4155)	A	4679	(4305;5053)	BC	*< 0.001*	4380	(3993;4766)	B	*0.037*
NaF-TP	14	3523	(3309;3736)	A	3588	(3191;3985)	C	0.627	3470	(3044;3895)	C	0.813
Overall	97	3851	(3762;3939)		4485	(4289;4680)			4478	(4222;4733)		
Intervention	*n*	LD_B (µm)			LD_{E1} (µm)			pe1	LD_{E2} (µm)			pe2
NT	19	194	(181;206)	A	245	(223;268)	B	*< 0.001*	280	(262;297)	C	*< 0.001*
NaF-Gel$_1$	15	198	(184;212)	A	200	(175;225)	C	0.830	129	(119;139)	D	*< 0.001*
AmF-Gel	17	188	(172;205)	A	169	(147;191)	C	0.059	153	(133;173)	D	*0.004*
NaF-Gel$_2$	15	192	(175;210)	A	157	(142;173)	C	*0.002*	139	(121;156)	D	*< 0.001*
KF-Gel	17	188	(172;204)	A	295	(271;319)	A	*< 0.001*	344	(318;371)	B	*< 0.001*
NaF-TP	14	164	(142;186)	A	316	(290;341)	A	*< 0.001*	408	(380;436)	A	*< 0.001*
Overall	97	188	(182;194)		230	(216;245)			242	(220;265)		

Means (95% confidence interval) of mineral losses (ΔZ) and lesion depths (LD) before (ΔZ_B, LD_B) and after pH cycling (7 days: ΔZ_{E1}, LD_{E1}; 14 days: ΔZ_{E2}, LD_{E2}) as well as changes in mineral loss ($\Delta\Delta Z_{E1}/\Delta\Delta Z_{E2}$) and lesion depth ($\Delta LD_{E1}/\Delta LD_{E2}$) for all groups. Italicized *p* values indicate significant differences in mineral losses and lesion depths before and after pH cycling (7 days: p_{E1}; 14 days: p_{E2}) (two-tailed paired *t* test)

Glandosane has a rather low pH, and its demineralizing effect has already been demonstrated in several in vitro studies [11, 15]. Recently, it could be shown that the demineralizing effect of Glandosane could be nearly completely inhibited when NaF-gel$_1$ (12,500 ppm F^-) or NaF-TP (5000 ppm F^-) were applied twice daily [16]. Nonetheless, in the previous studies, specimens were solely stored in Glandosane and no additional demineralization and nighttime period were established. Thus, so far, only the best case scenario (without demineralizing periods) was simulated. Contrastingly, in the present study, re- and demineralizing periods were included resulting in a more realistic model. Under these conditions, further demineralization was observed for the use of Glandosane alone as observed previously, as well. Thus, the demineralizing potential could even be induced with a rather short contact time and alongside neutral periods. This result should lead to rethinking of the widespread use of Glandosane.

In order to mimic the oral environment of patients suffering from xerostomia, the pH cycling protocol was slightly altered compared to previous pH cycling studies [25–27]. Firstly, the demineralizing phases were prolonged; instead of 30 min [26, 27], demineralization phases lasted 60 min. Secondly, specimens were consecutively subjected to a demineralizing and

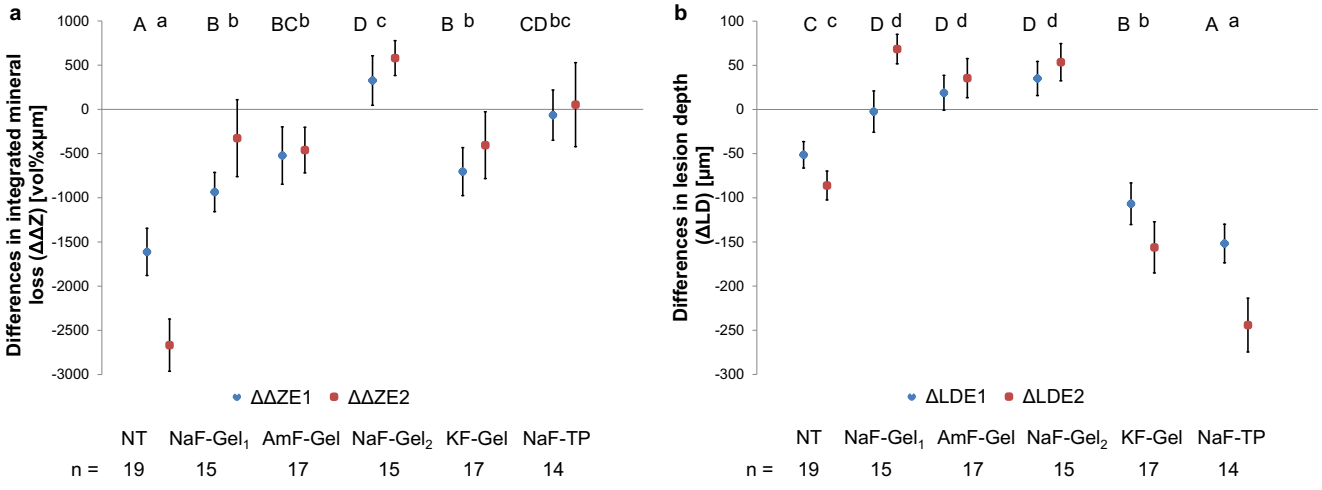

Fig. 2 Means (95% confidence intervals) of the changes in mineral loss ($\Delta\Delta Z_{E1}/\Delta\Delta Z_{E2}$) and lesion depth ($\Delta LD_{E1}/\Delta LD_{E2}$). Different letters indicate significant differences between treatment groups for each storage time (uppercase letters $\Delta\Delta Z_{E1}/\Delta LD_{E1}$; lowercase letters $\Delta\Delta Z_{E2}/\Delta LD_{E2}$) ($p < 0.05$, Bonferroni post hoc test) [rhombus $\Delta\Delta Z_{E1}/\Delta LD_{E1}$; square $\Delta\Delta Z_{E2}/\Delta LD_{E2}$]

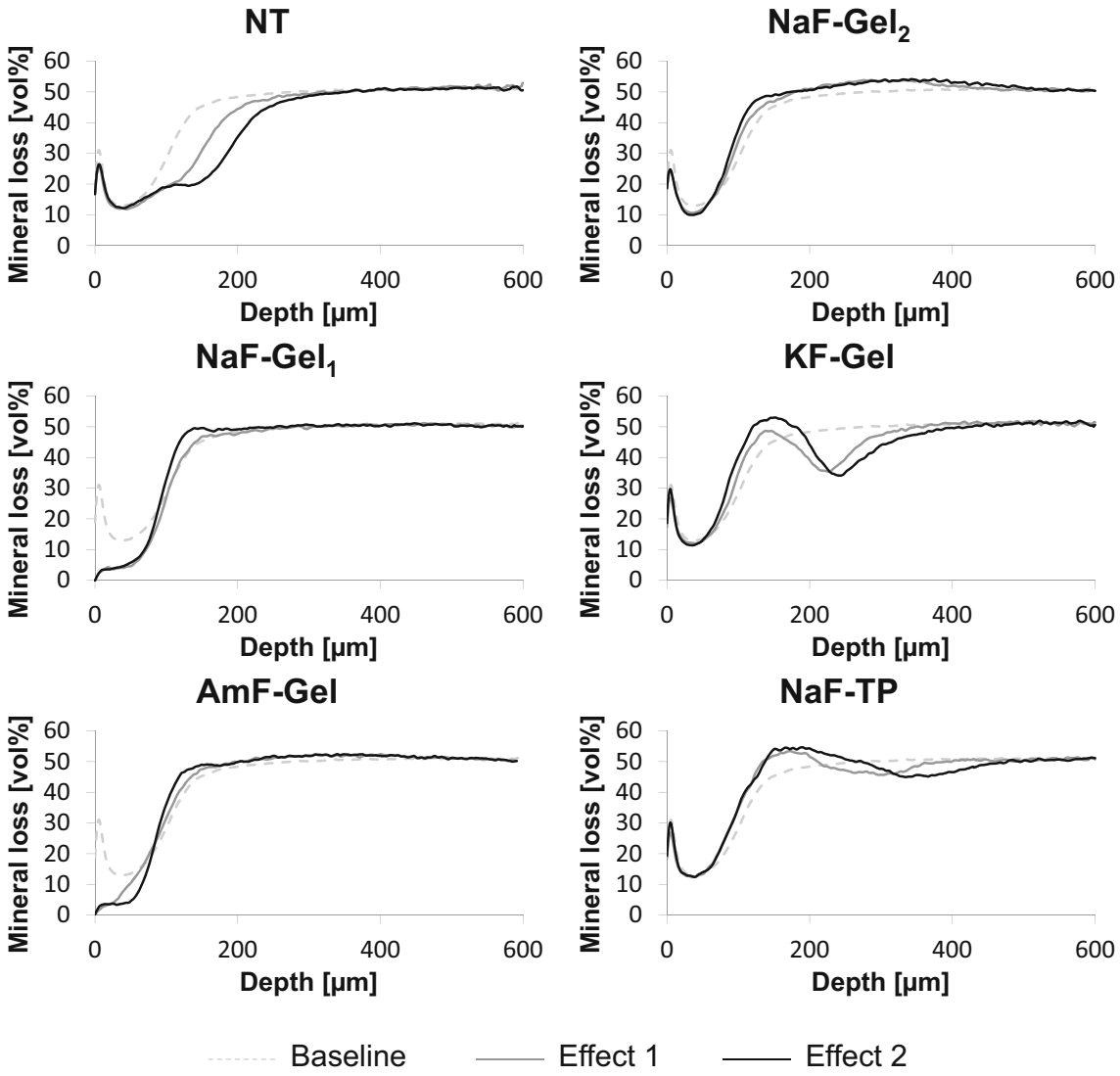

Fig. 3 Mean mineral density profiles of pre-demineralized dentin surfaces (baseline) and after 7 days pH cycling (Effect1) and 14 days cycling (Effect2) using the TMR/T-WIM calculation program

(potentially demineralizing) saliva substitute. Furthermore, to mimic a neutral phase during bedtime, specimens were stored in 100% humidity in the meantime. Although this design seems to be very extreme when compared to the in vivo situation, it simulates the reduced oral clearance rate of patients suffering from hyposalivation [28] since pH neutralization after a cariogenic attack and after the application a (potentially demineralizing) saliva substitute is slowed down. Therefore, it might also be speculated that effects of the fluoride agents might be less influential on mineralization when using a neutral or remineralizing saliva substitute.

Furthermore, the additional use of the tested fluoride agents significantly hampered further lesion progression when compared with no additional treatment. Interestingly, only the additional use of NaF-gel$_2$ induced a significant gain in mineral content. Although NaF-gel$_2$ was not tested in one of the previous models, the results seem to be in agreement with a previous in vitro study on erosion [29]. Under erosive conditions, NaF-gel$_2$ (as well as AmF-gel) demonstrated a significantly higher anti-erosive effect compared with no treatment.

Several studies demonstrated that the effect of fluoride agents might be increased by reducing their pH [30–32]. For acidic fluoride agents, the formation of calcium fluoride is enhanced compared with neutral ones. Furthermore, the adsorption of mineral ions into the lesion increases with decreasing pH. Thus, the remineralizing effect of acidic agents is supposed to be significantly higher than the effect observed for neutral agents [30]. However, in the present study, the acidic gels (AmF-gel and NaF-gel$_1$) could only hamper further demineralization. Contrastingly, only the neutral NaF-gel$_2$ demonstrated a significant gain in mineral content. Therefore, it might be speculated that in the present study,

the beneficial effect of the rather low pH of the fluoride agent (being supposed to increase mineral diffusion) was superimposed by the low pH of the saliva substitute Glandosane. Therefore, the rather low pH of a fluoride agent might not necessarily be required to increase mineral gain under these circumstance. In consequence, it might also be speculated that when acidic products are already used, neutral fluoride agents should be preferred.

After pH cycling, a significantly lower lesion progression was observed for specimens treated with NaF-gel$_1$ when compared with no treatment. However, when compared to NaF-gel$_2$ and NaF-TP, a less pronounced remineralizing effect was observed. The less pronounced remineralizing may, firstly, be based on the different pH values of the fluoride agents (as discussed above) or, secondly, caused by the Carbopol polymer (carbomer 956). Carbomer 956 is incorporated in NaF-gel$_1$ but not in the other fluoride agents [11]. In NaF-gel$_1$, it is used as thickening agent. Although the formation of calcium fluoride (CaF_2) on the dentin surface has not been analyzed in the present study, it might be speculated that Carbomer 956 has also bound CaF_2 just being incorporated in the enamel surfaces after applying the fluoride agent [33]. This "temporarily bound layer" was presumably removed during rinsing procedure, not being bioavailable during the following demineralization period. Consequently, this resulted in further surface mineral loss.

Glandosane in combination with the acidic AmF-gel presented a significantly lower lesion progression than Glandosane alone. This is in agreement with a previous in situ study [34]. After treatment with AmF-gel, the fluoride uptake was significantly higher when compared with a 5000 ppm F^- gel and a fluoride-free placebo gel. Furthermore, two in vitro studies on erosion [29, 35] concluded that the additional use of a highly fluoridated acidic AmF-gel may protect enamel against erosion. Nevertheless, when compared to the NaF-gel$_2$ and NaF-TP, a less pronounced mineral gain for the AmF-gel was observed. The low pH of the AmF-gel seemed to have no additional effect if demineralizing conditions predominate (as discussed above).

In the present study, NaF-TP and KF-gel significantly hampered further demineralization (KF-gel) or induce slight remineralization (NaF-TP). Furthermore, specimens of both agents showed an intact surface layer after pH cycling. Nevertheless, a second lesion body (lamination) after pH cycling for 7 as well as 14 days could be shown in both groups. In general, laminated (or layered) lesions present different surface zones with different mineral content [36]. The incorporation of fluorides seem to cause larger and less soluble crystallites [37]. Additionally, the fluorohydroxyapatites in the surface layer decrease the buffer capacity compared with hydroxyapatite [37]. Consequently, acids can easily pass the crystal structure of the original lesion without further neutralization [15, 26] resulting in a second lesion body. Interestingly, lamination has been observed in several pH cycling studies on enamel specimens [26, 27, 38] as well as dentin specimens [15]. However, lamination characteristics varied widely. In one pH cycling model, lamination was only observed for dentifrices containing 2800 ppm F^- [38]. Contrastingly, an inverse correlation between fluoride concentration and severity of the lamination was observed in other models [15, 26, 27].

Within the limitations of this in vitro study, it can be concluded that all fluoride agents could significantly hamper the adverse effects of a demineralizing saliva substitute. However, slight mineral gain was only observed for the neutral NaF-gel$_2$ (12,500 ppm F^-) as well as 5000 ppm F^- toothpaste. Further in vitro studies need to improve our knowledge about potentially demineralizing saliva substitute and the risks of long-term use.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interests.

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

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

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