

Saliva Substitutes in Combination with Highly Concentrated Fluorides and Brushing: In vitro Effects on Enamel Subsurface Lesions

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Key Words

Demineralisation · Enamel · Fluoride gel · Microradiography · Octacalcium phosphate · Remineralisation · Saliva substitute · Subsurface lesion · Toothpaste

Abstract

Hyposalivation is often associated with high caries activity, in particular in patients undergoing irradiation in the head/neck area. Besides the use of saliva substitutes to relieve the oral symptoms, daily application of fluoride gels or toothpaste (5,000 µg F⁻/g) is recommended for caries prevention. The aim of this study was to evaluate potentially remineralising effects of these fluoride agents in combination with saliva substitutes on enamel subsurface lesions. Demineralised bovine specimens were either stored in mineral water [control; saturation with respect to octacalcium phosphate (S_{OCP}): 0.8], a demineralising saliva substitute (Glandosane; S_{OCP} : 0.3) or in a modified (with respect to S_{OCP}) saliva substitute [Saliva natura (SN); S_{OCP} : 1.9] for 5 weeks (37°C). The following treatments were applied twice daily (11–13/group): no treatment (0), ProSchmelz fluoride gel (PS; 10 min application), Duraphat toothpaste (DP; 10 s; brushing with toothpaste/storage solution slurry), combination of DP+PS. Mineral pa-

rameters before/after storage were evaluated from microradiographs. Storage in Glandosane led to significant demineralisation ($p < 0.05$; paired t test), whereas additional use of fluoride agents neutralised the demineralising effect ($p > 0.05$). Storage in water alone resulted in no changes in mineral parameters ($p > 0.05$), whereas in combination with fluorides remineralisation could be shown ($p < 0.05$). For SN alone, remineralisation was observed ($p < 0.05$), but no additional beneficial effects of fluorides were detected. Under the conditions chosen, the fluoride agents reduce the demineralising effects of Glandosane and promote the remineralisation of specimens stored in water. Remineralising effects of SN could not be enhanced by the fluorides.

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Patients suffering from hyposalivation arising from irradiation in the head and neck area frequently experience high caries activity [Atkinson et al., 2005; Kielbassa et al., 2006]. For caries prevention, the daily application of fluoride agents in combination with meticulous oral hygiene and the careful control of diet are recommended. Saliva substitutes are most frequently applied to relieve the sensation of dry mouth [Epstein et al., 1996; Vissink et al., 2003].

Some commercially available saliva substitutes have demonstrated neutral or remineralising effects, and others have been shown to substantially demineralise enamel and dentin [Joyston-Bechal and Kidd, 1987; Smith et al., 2001; Meyer-Lueckel et al., 2002]. To increase remineralising capacities, fluoride, calcium and phosphate have been added to saliva substitutes, leading to solutions that are supersaturated with respect to the relevant calcium phosphates, such as octacalcium phosphate (OCP) and hydroxyapatite (HA). As a result of the use of these experimental saliva substitutes, remineralisation has been observed in vitro [Tschoppe et al., 2009b; Meyer-Lueckel et al., 2010].

For caries prevention, in addition to the regular use of fluoride toothpaste, highly fluoridated products such as gels are commonly recommended for irradiated patients [Nieuw Amerongen and Veerman, 2003]. Highly concentrated fluoride toothpaste seems to have a beneficial effect on the rehardening of root caries [Lynch and Baysan, 2001]. However, patients often demonstrate poor compliance with these regimens [Horiot et al., 1983; Epstein et al., 1996]. Preferably, a remineralising saliva substitute could manage both outcomes (dental caries and hyposalivation) while avoiding the additional use of highly concentrated fluoride agents (e.g. gels or special toothpastes).

The aim of this in vitro study was to evaluate the effects of daily applications of a fluoride gel and/or brushing with toothpaste (with a high fluoride content of 5,000 µg F⁻/g) on demineralised bovine enamel in combination with 2 saliva substitutes using a remineralisation model. Because a commercially available, potentially demineralising saliva substitute (Glandosane) is still commonly used, this product was compared with a potentially remineralising solution [modified in saturation with respect to the relevant calcium phosphates (Saliva natura, SN; Medac, Hamburg, Germany)]. Mineral water was used as a control. We hypothesised that the additional use of a fluoride agent in combination with the demineralising saliva substitute (Glandosane) would result in a significantly less pronounced demineralisation effect and that using the remineralising saliva substitute (modified SN) would result in significantly enhanced remineralisation.

Materials and Methods

Specimen Preparation

Eighty recently extracted permanent bovine central incisors were used. From each crown, 2 specimens (6 × 4 × 4 mm³) from the labial aspect were prepared under running tap water using a diamond-coated band saw (Exakt 300cl; Exakt Apparatebau,

Norderstedt, Germany). Subsequently, the 160 enamel specimens were embedded in epoxy resin (Technovit 4071; Heraeus Kulzer, Wehrheim, Germany), and the natural surface was kept free from resin. The specimens were ground flat and hand-polished to 4,000 grit (silicon carbide; Struers, Copenhagen, Denmark), thereby removing the outer parts of the enamel (approx. 200 µm).

One quarter of each specimen's surface was covered with an acid-resistant nail varnish (Lycra Flex + Silk; Astor, Paris, France) to serve as a sound enamel control. Artificial lesions were prepared by immersion in 5 litres of a solution containing 6 µmol/l methylhydroxydiphosphonate, 3 mmol/l CaCl₂·2H₂O, 3 mmol/l KH₂PO₄ and 50 mmol/l CH₃COOH (Merck, Darmstadt, Germany) at pH 4.95 in an incubator (37°C; BR 6000; Heraeus Kulzer) for 15 days. The pH value was monitored daily (pH electrode GE 100 BNC connected to pH meter GMH 3510; Greisinger, Regenstauf, Germany), and slight elevations were corrected with hydrochloric acid to maintain the pH between 4.92 and 4.98 during the demineralisation period. Standard buffer solutions (Sigma-Aldrich, Steinheim, Germany) with nominal pH values of 4.0 and 7.0 ± 0.01 units were used to calibrate the pH meter. After the demineralisation period, 4 specimens were evaluated using transversal microradiography to control the outcome of the demineralisation process (i.e. the creation of subsurface lesions).

Preparation of Experimental Solutions

For aqueous solutions, the degrees of saturation with respect to the caries-relevant compounds (e.g. dicalcium phosphate dihydrate, OCP, HA, calcium fluoride and fluoroapatite) can be calculated if the pH and the concentrations of certain ions are known (table 1). Furthermore, using the software developed by Shellis [1988], the ionic strength has been included in the calculation of the degrees of saturation. The calcium-to-phosphate ratio of the modified SN (polysaccharide-based) was determined to be 1:1.6. The natural calcium and phosphate of the unmodified SN solution [Tschoppe et al., 2009a] and the further addition of calcium (as CaCl₂·2H₂O) and phosphate (as K₂HPO₄/KH₂PO₄) resulted in a supersaturated solution with respect to the relevant calcium phosphates at the buffered pH of 5.95 (table 1). Preliminary tests revealed that a K₂HPO₄/KH₂PO₄ ratio (phosphate buffer) of 1:2 was optimal for maintaining a constant pH (all chemicals were from Merck). Additionally, 0.1 mmol/l sodium fluoride (Fisher Scientific, Ulm, Germany) was added to the SN. After preparation, modified SN tended to precipitate slightly. Glandosane (carboxymethylcellulose-based; Cell Pharm, Hannover, Germany), representing a demineralising saliva substitute [Joyston-Bechal and Kidd, 1987; Meyer-Lueckel et al., 2002], was tested (table 1). In addition to these 2 saliva substitutes, a non-carbonated mineral water (Volvic; Danone, Frankfurt, Germany) of pH 7.16 was also used.

Specimen Treatment

After the demineralisation period, half of each demineralised surface was covered with nail varnish (to act as control). The specimens were then randomly divided into 3 groups and stored in either non-carbonated mineral water, Glandosane or SN for 5 weeks (37°C). Moreover, the specimens were subjected to one of the following treatments twice daily (table 2): no treatment (0); the gentle application of ProSchmelz fluoride gel (PS; GSK, München, Germany) for 10 min; brushing gently for 10 s with Duraphat (DP; 5,000 µg F⁻/g, Colgate-Palmolive, Hamburg, Germany) tooth-

Table 1. Compositions of the control solution (water) as well as the saliva substitutes and calculated saturations with respect to calcium phosphates and CaF₂

Solution	Calculated saturation of an aqueous solution with respect to					pH	Ion concentrations, mmol/l						
	DCPD	OCP	HA	CaF ₂	FA		Ca	PO ₄	F	K	Cl	Na	Mg
Water (Volvic)	0.2	0.8	4.9	–	–	7.16	0.3	0.2	0	0.2	0.4	0.5	0.3
Glandosane	0.2	0.3	1.0	–	–	5.35	1.0	2.6	0	19.0	33.8	14.8	0.3
Modified SN	1.3	1.9	7.1	0.6	28.5	5.95	3.2	5.0	0.1	6.7	6.3	0	0

DCPD = Dicalcium phosphate dihydrate; OCP = octacalcium phosphate; HA = hydroxyapatite; CaF₂ = calcium fluoride; FA = fluoroapatite.

Table 2. Fluoride agents and their treatment regimes

Agents	Code	Concentration µg F ⁻ /g	pH	Treatment
None	0	–	–	no treatment
ProSchmelz fluoride gel ¹	PS	12,500	6.03	contact with the gel for 10 min in the morning and evening
Duraphat toothpaste	DP	5,000	DP/water (1:3) – 8.15 DP/G (1:3) – 7.54 DP/SN (1:3) – 7.61	slurry (ratio 1:3) from toothpaste with the respective storage solution and brushing for 10 s with a total contact time of 130 s
Duraphat toothpaste + ProSchmelz fluoride gel	DP+PS	see DP and PS	see DP and PS	same as group DP and additional contact with the gel for 10 min in the morning and evening after the toothpaste procedure

After each treatment, the specimens were washed for 20 s with approximately 20 ml deionized water (n = 11–13/subgroup). Duraphat toothpaste was diluted 1:3 with storage solution to obtain a homogeneous slurry (EN ISO 11609), and the specimens were brushed gently with an electric toothbrush (Oral-B Professional Care 7500 with brush head Oral-B Flexisoft). G = Glandosane.

¹ Contains 0.256 mol/l phosphate, as analysed in a previous study [Meyer-Lueckel and Tschoppe, 2010].

paste/storage solution slurry, with an additional contact time of 120 s with the slurry (total contact time, 130 s), or a combination of treatments DP and PS (DP+PS). After 2 weeks, half of the exposed surfaces were nail varnished (effect after 2 weeks). Every second day, the solutions of each group were replenished (24 ml each time, 37°C), and the pH values of the storage solutions were checked. The pH values remained stable during the entire experimental period of 5 weeks.

Transversal Microradiography

After in vitro exposure, thin sections (100 µm) were prepared, and contact microradiographs of the enamel specimens were obtained by transversal microradiography, which were then analysed using special software (transversal microradiography for Windows 2.0.27.2; Inspektor Research System BV, Amsterdam, the Netherlands). For full details, see Meyer-Lueckel et al. [2010]. The investigator was blind with respect to the group allocation.

Mineral density profiles were evaluated from which integrated mineral loss (ΔZ) and lesion depth (LD) values were calculated following initial demineralisation (ΔZ_{Demin}, LD_{Demin}) and after exposure to the various test solutions for either 2 (ΔZ_{Effect 2}, LD_{Effect 2}) or 5 weeks (ΔZ_{Effect 5}, LD_{Effect 5}), respectively. Each pair of values was corrected by subtracting the respective values for sound enamel (ΔZ_{Sound} and LD_{Sound}) before data analysis. Changes in mineral loss (ΔΔZ₂ = ΔZ_{Demin} – ΔZ_{Effect 2}, ΔΔZ₅ = ΔZ_{Demin} – ΔZ_{Effect 5}) and in LD (ΔLD₂ = LD_{Demin} – LD_{Effect 2}, ΔLD₅ = LD_{Demin} – LD_{Effect 5}) were analysed for treatment differences. Positive and negative values of ΔΔZ or ΔLD indicated net remineralisation and net demineralisation, respectively.

Statistical Analyses

Statistical analyses were performed using a statistical software package (PASW for Windows, version 18.0; SPSS, Munich, Germany). Data were tested for normal distribution (Kolmogorov-

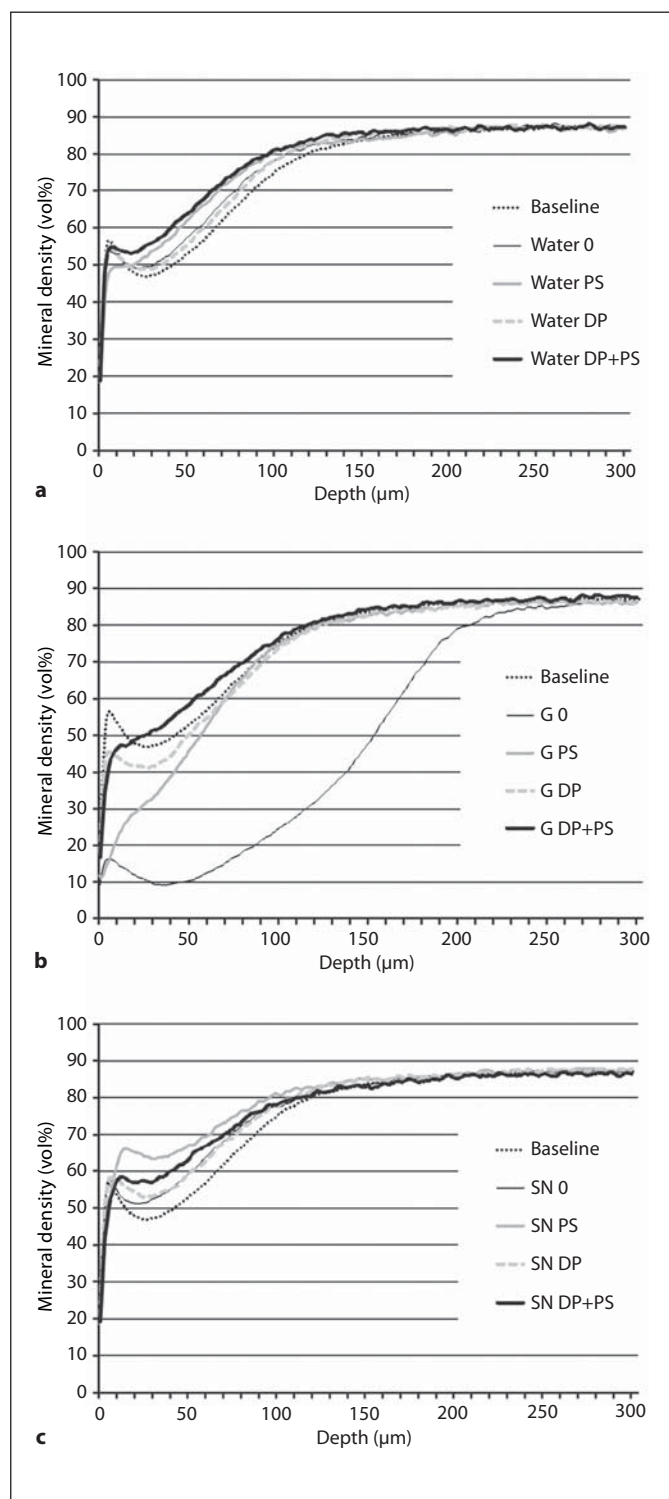


Fig. 1. Mean mineral density profiles of the enamel specimens after 5 weeks of storage in water (**a**), Glandosane (G; **b**) and modified SN (**c**) with or without additional treatment with fluoride agents compared to baseline. For each storage solution the respective subgroups are depicted: no treatment (0), PS fluoride gel, DP toothpaste, combination DP+PS.

Smirnov test). $\Delta\Delta Z$ and ΔLD were analysed with regard to the variables 'solution' and 'treatment' using two-way ANOVA. One-way ANOVA was applied to test for significant differences of $\Delta\Delta Z$ and ΔLD between the groups. Analysis of variance was followed by a Tukey post hoc test. Differences in mineral loss and LD before and after storage/treatment were compared by the paired t test with the level of significance set at 5%.

Results

All the in vitro demineralised specimens developed subsurface lesions, consistently revealing a surface layer that was more mineralised than the body of the lesion with no signs of erosive loss. ΔZ as well as LD did not differ significantly between the various groups at baseline ($p = 0.421$ and $p = 0.175$, respectively; ANOVA). Two specimens were lost during preparation resulting in 11 specimens/group for SN-DP and 13 specimens for all other groups. Mineral losses ($\Delta Z_{\text{Effect 5}}$, $\Delta Z_{\text{Effect 2}}$) and lesion depths ($LD_{\text{Effect 2}}$, $LD_{\text{Effect 5}}$) were significantly affected by 'solution' and 'treatment' ($p < 0.006$, two-way ANOVA). Furthermore, significant interaction could be observed among these factors for $\Delta Z_{\text{Effect 5}}$, $\Delta Z_{\text{Effect 2}}$ and $LD_{\text{Effect 5}}$ ($p < 0.001$) but not for $LD_{\text{Effect 2}}$ ($p = 0.575$).

Storage in Glandosane, either without further treatment or in combination with PS, resulted in pronounced erosion of the surface layer. For Glandosane-PS and Glandosane-DP, inhibition of demineralisation of deeper lesion parts could be observed. Specimens stored in Glandosane and additionally treated with DP+PS revealed nearly complete inhibition of demineralisation of the surface layer; for deeper lesion parts, a tendency towards remineralisation could be seen. Specimens of all other groups revealed subsurface lesions without erosive or abrasive loss after storage (fig. 1).

In vitro exposure for either 2 or 5 weeks to Glandosane alone resulted in significantly lower $\Delta\Delta Z_{\text{Effect 2}}$, $\Delta\Delta Z_{\text{Effect 5}}$ and $\Delta LD_{\text{Effect 5}}$ values compared to those for all other solutions ($p < 0.05$; ANOVA, Tukey). $\Delta LD_{\text{Effect 2}}$ values of specimens stored in Glandosane were significantly lower compared to those for water-PS, water-DP and water-DP+PS ($p < 0.05$) but revealed no significant differences compared to the other groups ($p > 0.05$). No significant differences with regard to $\Delta\Delta Z_{\text{Effect 2}}$, $\Delta\Delta Z_{\text{Effect 5}}$, $\Delta LD_{\text{Effect 2}}$ and $\Delta LD_{\text{Effect 5}}$ values could be observed between only storage and storage plus any of the treatments for water and SN ($p > 0.05$; fig. 2).

Remineralisation (all outcomes, i.e. decreased ΔZ and LD) could be observed for water in combination with all fluoride treatments after both periods ($p < 0.05$; paired

t test). After 2 as well as 5 weeks of exposure, significantly increased (demineralisation) ΔZ ($\Delta Z_{\text{Effect 2}}$ and $\Delta Z_{\text{Effect 5}}$) as well as LD ($LD_{\text{Effect 2}}$ and $LD_{\text{Effect 5}}$) compared with baseline could be observed for Glandosane alone ($p < 0.05$; paired t test). Specimens of SN-0 and SN-PS showed significantly decreased $\Delta Z_{\text{Effect 5}}$ values compared with baseline ($p < 0.001$). For SN-DP, this was the case for $\Delta Z_{\text{Effect 2}}$, $\Delta Z_{\text{Effect 5}}$ and $LD_{\text{Effect 5}}$ (tables 3, 4).

Discussion

This in vitro study demonstrated mainly that both fluoride gel (PS) and fluoride toothpaste (DP) reduce the detrimental demineralising effect of Glandosane observed previously [Joyston-Bechal and Kidd, 1987; Smith et al., 2001; Meyer-Lueckel et al., 2002]. The modified (with respect to the saturation of calcium phosphates) SN alone and in combination with the fluoride gel or toothpaste results in remineralising effects after 5 weeks. Therefore, the first hypothesis (that the additional use of fluoride results in less pronounced demineralisation when the specimens are stored in Glandosane) could not be rejected, whereas the second hypothesis (that the additional use of fluoride results in enhanced remineralisation when modified SN is used) could be rejected.

In the present study, the demineralised specimens (subsurface lesions) were stored daily for a period of 5 weeks in the different solutions. This can be considered as an extremely long contact time with artificial saliva. However, it is important to consider that patients suffering from hyposalivation generally use saliva substitutes or non-carbonated water ad libitum [Atkinson et al., 2005; Kielbassa et al., 2006]. Thus, similar effects might be conceivable after longer in vivo periods. No pH cycling model was chosen to evaluate the potential remineralising effects of the fluoride agents more efficiently. However, this should be taken into account when interpreting the data.

For patients who are currently undergoing or have undergone radiation therapy in the past, brushing with a highly concentrated fluoride toothpaste and the application of a fluoride gel using a customised mouth tray are recommended more frequently compared to the regimes recommended for persons with normal salivary flow rates [Wei and Yiu, 1993]. Mimicking this scenario, each specimen was either brushed with a paste/saliva substitute slurry or a paste/water slurry for 10 s twice daily (total contact time, 130 s), or the gel was applied gently to the top surface of each specimen for 10 min twice daily.

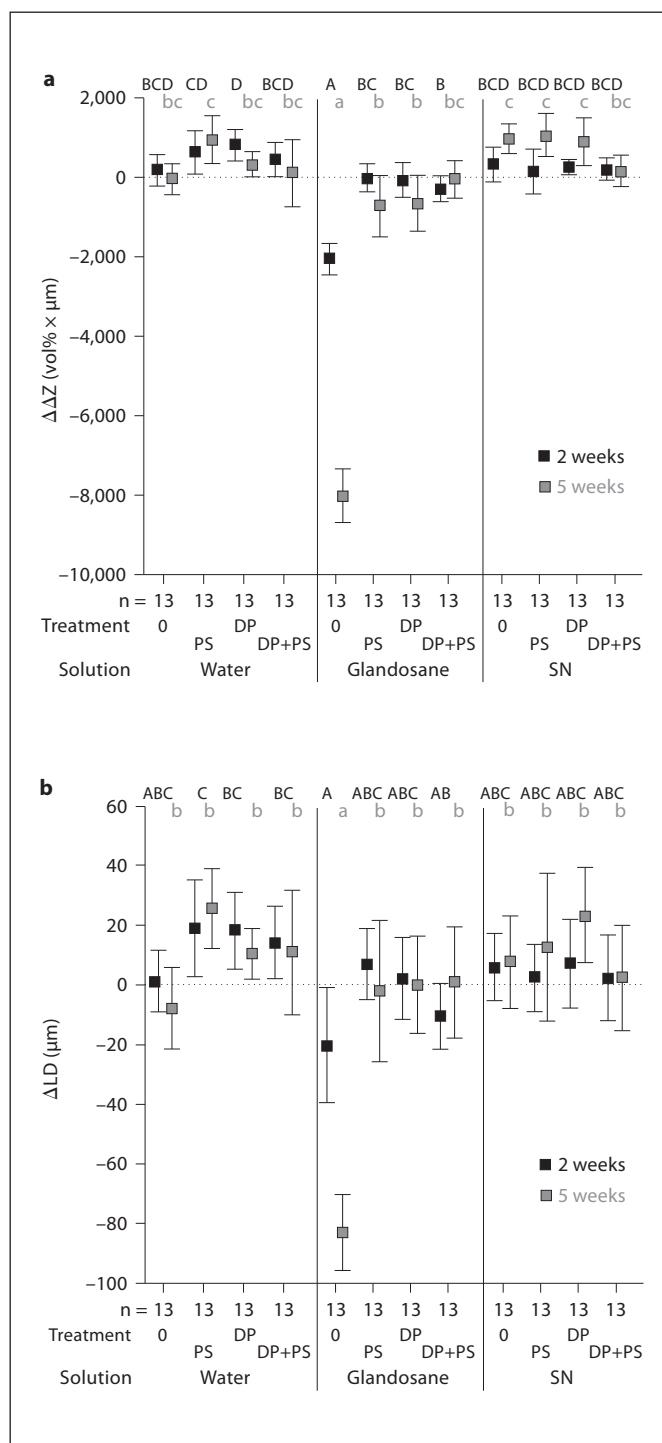


Fig. 2. Means and 95% confidence intervals of the differences in mineral change (a) and LD (b) after 2 and 5 weeks storage in the various solutions (water, Glandosane and modified SN). Within each main group (solution), specimens were additionally treated as follows: no treatment (0), PS fluoride gel, DP toothpaste, combination DP+PS. Different letters at the top indicate significant differences between the subgroups within each storage period ($p < 0.05$; ANOVA, Tukey post hoc test).

Table 3. Means and standard deviations of the mineral losses (ΔZ ; vol% $\times \mu\text{m}$) after in vitro demineralisation (ΔZ_{Demin}) and storage in the various solutions with and without additional treatment with fluoride agents for 2 ($\Delta Z_{\text{Effect 2}}$) or 5 weeks ($\Delta Z_{\text{Effect 5}}$)

Solution	Treatment	ΔZ_{Demin}		$\Delta Z_{\text{Effect 2}}$		Demin-Effect 2 P	$\Delta Z_{\text{Effect 5}}$		Demin-Effect 5 P
		mean	SD	mean	SD		mean	SD	
Water	0	2,824	806	2,633	864	0.319	2,846	874	0.907
	PS	3,224	1,127	2,582	825	<i>0.024</i>	2,280	949	<i>0.005</i>
	DP	3,250	932	2,419	638	<i>0.001</i>	2,917	962	<i>0.045</i>
	DP+PS	2,561	510	2,012	734	<i>0.002</i>	2,020	991	<i>0.008</i>
Glandosane	0	2,853	861	4,933	659	<i><0.001</i>	10,868	1,536	<i><0.001</i>
	PS	3,284	646	3,326	853	0.801	4,028	1,176	0.056
	DP	2,930	1,176	3,009	861	0.689	3,605	1,236	0.051
	DP+PS	2,935	1,235	3,247	1,330	0.057	2,997	1,397	0.773
Modified SN	0	3,464	835	3,158	649	0.148	2,495	911	<i><0.001</i>
	PS	2,992	969	2,862	1,320	0.616	1,954	597	<i>0.002</i>
	DP	3,158	1,224	2,898	1,233	<i>0.010</i>	2,275	925	<i>0.007</i>
	DP+PS	2,678	775	2,497	704	0.202	2,535	703	0.448

p values of differences between the values after demineralisation and storage/treatment for either 2 (Demin-Effect 2) or 5 weeks (Demin-Effect 5) within each group (n = 11 in group SN-DP and n = 13 in all other groups) as analysed by t tests. p values of pairs differing significantly are italicised.

Table 4. Means and standard deviations of the LD (μm) after in vitro demineralisation (LD_{Demin}) and storage in the various solutions with and without additional treatment with fluoride agents for 2 ($\text{LD}_{\text{Effect 2}}$) as well as 5 weeks ($\text{LD}_{\text{Effect 5}}$)

Solution	Treatment	LD_{Demin}		$\text{LD}_{\text{Effect 2}}$		Demin-Effect 2 P	$\text{LD}_{\text{Effect 5}}$		Demin-Effect 5 P
		mean	SD	mean	SD		mean	SD	
Water	0	102	22	101	23	0.810	110	20	0.224
	PS	118	25	99	28	<i>0.027</i>	92	32	<i>0.001</i>
	DP	113	24	94	16	<i>0.009</i>	102	19	<i>0.020</i>
	DP+PS	100	25	85	19	<i>0.048</i>	83	27	<i>0.043</i>
Glandosane	0	106	21	126	22	<i>0.041</i>	189	21	<i><0.001</i>
	PS	120	18	113	27	0.220	122	35	0.851
	DP	118	29	116	17	0.756	118	26	0.998
	DP+PS	112	22	123	19	0.057	111	32	0.915
Modified SN	0	121	17	115	13	0.265	113	26	0.305
	PS	111	25	109	27	0.637	99	28	0.287
	DP	122	23	114	37	0.311	98	26	<i>0.008</i>
	DP+PS	121	28	119	21	0.747	118	22	0.760

p values of differences between the values after demineralisation and storage/treatment for either 2 (Demin-Effect 2) or 5 weeks (Demin-Effect 5) within each group (n = 11 in group SN-DP and n = 13 in all other groups) as analysed by t tests are given. p values of pairs differing significantly are italicised.

Although not standardised (i.e. by using a brushing machine), the chosen brushing procedure by itself should not have influenced the results (i.e. the group comparison) because a variable toothbrush pressure should have averaged out over time. Furthermore, the evaluation of the surface during the transversal microradiography assessment indicated no visible abrasion of the surface arising from the brushing procedure.

Saliva substitutes for patients with diminished saliva production have been developed primarily to alleviate the symptoms experienced during mastication, swallowing and speech [Atkinson et al., 2005]. With reference to these parameters, saliva substitutes have been shown to provide relief in cases of radiogenic hyposalivation and elsewhere [Nieuw Amerongen and Veerman, 2003]. However, patients with hyposalivation often alleviate their oral symptoms by drinking water because it is convenient and cheap. For this reason, a fluoride-free, non-carbonated mineral water was used in this study as a control. After both storage periods in water, no changes in mineralisation were observed, which might be explained by the calculated saturations with respect to the relevant calcium phosphates (S_{HA} of 4.9/ S_{OCP} of 0.8) [Iijima, 2001].

The commercially available saliva substitute Glandosane demonstrated a remarkable demineralising effect in addition to the erosion of the surface. Similar effects on enamel have been observed previously [Joyston-Bechal and Kidd, 1987; Meyer-Lueckel et al., 2002]. This might be explained by the low pH value; the unspecified amount of titratable acids may have led to low saturation with respect to HA ($S_{HA} = 1.0$; table 1). In contrast, the modified SN remineralised the specimens, and this might be explained by the supersaturation with respect to HA, OCP and DCPD [Tschoeppe et al., 2009b].

Two different fluoride-containing agents were used in this study: a fluoride gel (12,500 $\mu\text{g F}^-/\text{g}$) and a highly concentrated fluoride toothpaste (5,000 $\mu\text{g F}^-/\text{g}$). All fluoride treatments were capable of preventing further demineralisation of the specimens stored in Glandosane. As a result of these fluoride treatments, a distinct calcium-fluoride-like layer on the specimen surfaces may have been established [Christoffersen et al., 1988; Ogaard, 2001], which appeared to be dissolved over time by Glandosane. Contact of the specimens with the neutral mineral water (S_{HA} of 4.9) should not have affected the layer mentioned above. Consequently, available fluorides in the layer were capable of remineralising the specimens [Ogaard, 2001], resulting in a more pronounced remineralisation compared to that associated with the use of mineral water alone.

PS fluoride gel, containing considerable amounts of phosphate [Meyer-Lueckel and Tschoeppe, 2010], demonstrated statistically significant remineralising effects when used in combination with SN and water without inducing erosion. Similar results have been observed in previous studies, in which even acidic fluoride gels have been shown to be effective in the prevention of erosion [Lagerweij et al., 2006]. One possible cause might be the pH value of PS, which was above the critical pH for enamel demineralisation [Featherstone, 2004]. The increased phosphate concentration may have also influenced the mineralisation process. If phosphate is present during formation of the calcium fluoride layer, it will be incorporated into the lattice structure. This 'phosphate-contaminated calcium fluoride' is understood to be more soluble than pure calcium fluoride [Christoffersen et al., 1988], leading to an enhanced transformation of this layer to fluoroapatite [Chander et al., 1982]. Consequently, the remineralisation of the deeper parts of the lesions and the surface could be observed when PS was used in combination with water or SN.

The application of DP toothpaste resulted in a remineralising effect when combined with SN or water. For both solutions, this may have been accomplished by the creation of a calcium-fluoride-like layer on the specimen surface. The brushing procedure with the slurry might have induced a slight abrasion on the outermost surface, resulting in a 'calcium-enriched slurry'. Because of the higher concentration of available fluoride when using DP compared with storage in water or SN alone, the formation of a calcium-fluoride-like layer was probably enhanced [Christoffersen et al., 1988; Cruz et al., 1992; Ogaard, 2001]. Thus, a remineralising effect was induced when using DP in combination with water. However, the remineralising effects observed when using SN alone were not enhanced.

SN was modified with fluoride, calcium and phosphate resulting in a solution that was supersaturated with respect to relevant calcium phosphates and calcium fluoride [Tschoeppe et al., 2009b]. Given that SN contains 2 $\mu\text{g F}^-/\text{g}$, it is no surprise that a precipitate was seen in the SN solution. Presumably this precipitate was fluoride-substituted apatite, though other phases are possible (e.g. dicalcium phosphate dihydrate). Hence, the remineralisation potential of the modified SN was slightly decreased. On preparing the slurry (by mixing SN with the fluoride toothpaste), the degree of saturation would have been increased even more and calcium-fluoride-like precipitates in the DP/SN slurry should have been favoured [Larsen and Jensen, 1994]. Such precipitates, together with the

calcium-fluoride-like layer mentioned above, could have caused surface blocking leading to reduced ion transport into deeper parts of the artificial lesions. Therefore, remineralisation induced by SN alone could not be enhanced using DP toothpaste. The combination of SN-DP+PS resulted in no significant remineralisation. Most likely, the calcium fluoride layer that we postulated to develop after brushing was enhanced by application of the fluoride gel, leading to a highly mineralised surface layer that blocked ion diffusion into deeper parts of the lesion during the subsequent storage in SN [Preston et al., 2008].

In patients with hyposalivation arising from irradiation in the head and neck area, long-term compliance with a 2-step regime is quite poor [Horiot et al., 1983; Epstein et al., 1996]. Therefore, the use of a remineralising saliva substitute alone could be a promising approach for dentate patients with hyposalivation to manage both outcomes (dental caries and hyposalivation). According to

the present results and using a 'remineralisation model', the modified SN, being supersaturated with respect to the relevant calcium phosphates, appears to have this potential. Within the limitations of this in vitro study, it can be concluded that the use of fluoride agents (i.e. a fluoride gel or highly concentrated fluoride toothpaste) reduces the detrimental effects of demineralising saliva substitutes, but it cannot enhance the remineralising effects of a remineralising artificial saliva.

Acknowledgements

This study was supported by the Deutsche Krebshilfe e.V. (grant 70-3117-Me-1).

Disclosure Statement

The authors declare no conflict of interest.

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