

# Stability against Brushing Abrasion and the Erosion-Protective Effect of Different Fluoride Compounds

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## Key Words

Abrasion · Brushing · Erosion · Fluoride

## Abstract

This study aimed to analyse the impact of brushing on the protective effect of different fluoride solutions on enamel and dentin erosion. Bovine enamel and dentin specimens were rinsed once with TiF<sub>4</sub>, AmF, SnF<sub>2</sub> (0.5 M F, 2 min) or water (control). Specimens were either left unbrushed or brushed with 10, 20, 50, 100 or 500 brushing strokes in an automatic brushing machine (2 N, non-fluoridated toothpaste slurry). Ten specimens per group were eroded with hydrochloric acid (HCl) (pH 2.3) for 60 s, and calcium release into the acid was determined by atomic absorption spectroscopy. Additionally, enamel and dentin surfaces were analysed by X-ray energy-dispersive spectroscopy (EDS) (n = 6/group) and scanning electron microscopy (SEM) (n = 2/group) before brushing and after 500 brushing strokes. Statistical analysis (p < 0.05) was performed by three- and one-way ANOVA (calcium release) or repeated measures ANOVA (EDS). TiF<sub>4</sub>, AmF and SnF<sub>2</sub> reduced the erosive calcium loss in unbrushed specimens to 58–67% (enamel) and 23–31% (dentin) of control. Calcium release increased with increasing brushing strokes prior to erosion and amounted to 70–88% (enamel) and 45–78% (dentin) of control after 500 brushing strokes. Brushing reduced the surface concentration of fluoride (AmF), tin (SnF<sub>2</sub>) and titanium (TiF<sub>4</sub>). SEM re-

vealed that surface precipitates were affected by long-term brushing. Brushing reduced the protective potential of TiF<sub>4</sub>, AmF and SnF<sub>2</sub> solutions. However, considering a small number of brushing strokes, the protective effect of fluoride solutions is only slightly affected by brushing abrasion.

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The efficacy of fluorides and fluoride-containing compounds to prevent dental erosion has been increasingly studied in the last years. While initial studies concentrated mainly on fluoride compounds forming CaF<sub>2</sub> precipitates on the surface, current research focuses on the efficacy of fluoride compounds containing polyvalent metal ions, such as tin, titanium or iron [Wiegand et al., 2008a; Schlueter et al., 2009c; Bueno et al., 2010]. The protective potential of the latter is attributed to the formation of acid-resistant surface coatings and an incorporation of fluoride and the respective ion in the hydroxyapatite lattice [Schlueter et al., 2009b; Wiegand et al., 2010b]. In this context it was also shown that polyvalent cations have a considerable anti-erosive effect even in the absence of fluoride [Sales-Peres et al., 2007; Ganss et al., 2008].

Several studies demonstrated a wide range in the efficacy of different fluoride compounds to prevent enamel and dentin erosion [Ganss et al., 2008; Schlueter et al., 2009a; Wiegand et al., 2009a]. Depending on the kind of fluoride compound, the formation of the surface precipi-

tates and, thus, the anti-erosive potential is related to the concentration and pH of the agent and the duration and frequency of application. While the acid resistance of fluoride precipitates was investigated in several studies [Attin et al., 2001; Ganss et al., 2007; Yu et al., 2010], only limited information about their abrasion resistance is available yet. This issue is of clinical interest, as dental hard tissues are usually exposed not only to erosive but also to abrasive influences, such as toothbrushing. Although toothbrushing with fluoridated toothpastes might reduce abrasion of eroded dental hard tissues compared to non-fluoridated toothpastes [Magalhaes et al., 2007, 2008], the use of fluoridated toothpastes alone had only a limited beneficial effect on dental erosion when compared to high-concentrated fluoride agents [Ganss et al., 2004a; Lagerweij et al., 2006]. Thus, fluoride precipitates which do not resist abrasive forces to some extent would require either a frequent application of the agent or the use of vehicles with a high adherence to the tooth surface, such as fluoride varnishes.

In a recent study it was shown that the anti-erosive effects of different fluoride toothpastes were decreased as soon as the specimens were not only immersed in but brushed with the respective toothpaste slurries [Ganss et al., 2011]. This finding might be related to the limited physical resistance of either the fluoride precipitates formed on the surface or the eroded surface itself, in case that incorporation of fluoride or other ions in the softened layer does not lead to complete rehardening [Wegehaupt et al., 2009; Ganss et al., 2011]. CaF<sub>2</sub> precipitates formed on enamel when sodium fluoride or amine fluoride is applied can be partially removed by brushing [Wegehaupt et al., 2009]. However, the ability of different fluoride compounds and complexes containing polyvalent metal cations to withstand brushing has not been systematically analysed yet. Therefore, it was the aim of the present study to investigate the influence of brushing on the erosion-protective effect of different fluoride solutions (AmF, TiF<sub>4</sub>, SnF<sub>2</sub>). The null hypothesis was that brushing will not increase the erosive calcium loss of enamel and dentin specimens treated with the different test solutions.

## Methods

### *Sample Preparation and Allocation to the Experiments*

Cylindric enamel and dentin specimens (3 mm in diameter, in total 304 enamel and 304 dentin specimens) were obtained with a water-cooled trephine bur from the crowns or roots, respectively, of freshly extracted, non-damaged bovine incisors, which were stored in 0.9% NaCl solution until used. The samples were embedded in acrylic resin (Paladur, Heraeus Kulzer, Hanau, Germany). Subse-

quently, enamel and dentin surfaces were ground flat and polished with water-cooled carborundum discs (1,200, 2,400 and 4,000 grit, Water Proof Silicon Carbide Paper, Struers, Erkrath, Germany), thereby removing approximately 200 µm of the outermost layer as verified with a micrometre (Digimatic, Mitutoyo, Tokyo, Japan).

Enamel and dentin specimens were randomly assigned to four groups (three test: TiF<sub>4</sub>, AmF, SnF<sub>2</sub>; one control: water) of 76 specimens each. 60 enamel and 60 dentin specimens were used for determination of erosive calcium loss and were randomly assigned to six subgroups (n = 10) accordingly to the number of brushing strokes (0, 10, 20, 50, 100 or 500 brushing strokes) applied before erosion. The remaining enamel and dentin specimens (each n = 16) were used for scanning electron microscopy (SEM) or X-ray energy-dispersive spectroscopy (EDS), which were performed after application of the respective test solution (SEM: each n = 2; EDS: each n = 6) and after application of 500 brushing strokes (SEM: each n = 2; EDS: each n = 6).

The brushing machine used consisted of six brushing chambers for two specimens each, allowing for brushing of up to 12 specimens in parallel in one experimental run. In one experimental run, all specimens in the brushing machine were brushed with the same amount of brushing strokes. The sequence of experimental runs in terms of applied brushing strokes was randomly determined. Six pairs of specimens (1 pair = 2 specimens from the same subgroup) were randomly selected from the different subgroups, pretreated with the respective solution and randomly assigned to the brushing chambers.

### *Test Solutions and Brushing Treatment*

Enamel and dentin specimens were treated once with equimolar solutions (0.5 M F) of TiF<sub>4</sub>, AmF, SnF<sub>2</sub> or with distilled water (control) for 2 min. The solutions were freshly prepared prior to application to the specimens. The pH of the solutions amounted to TiF<sub>4</sub>: 1.3, AmF: 4.3 and SnF<sub>2</sub>: 2.6 (Metrom 827 pH Lab, Metrom, Herisau, Switzerland). Specimens pretreated with TiF<sub>4</sub>, AmF or SnF<sub>2</sub> were then rinsed for 10 s with distilled water.

Brushing of the specimens was performed in an automatic brushing machine as described previously [Wiegand et al., 2008b, 2009b]. The toothbrushes (Paro M43, Esro AG, Thalwil, Switzerland, 0.2 mm filament diameter) were applied at 2 N brushing force with a brushing frequency of 120 strokes/min. Each two specimens from the same subgroup were brushed in the same brushing well with 4 ml toothpaste slurry (2 ml/specimen). The non-fluoridated toothpaste slurry was prepared by mixing 100 g of a baseline formulation (saliva substitute (79.2%) [Göhring et al., 2004], 85% glycerine (10%), 1.62% sodium bicarbonate (10.3%), carboxymethylcellulose (0.5%)) with 20 g calcium pyrophosphate (Fluka, Buchs, Switzerland, Lot: 88HO466). The RDA and REA value of the toothpaste slurry amounted to 50 and 6, respectively, and was determined according to Imfeld [2010]. After brushing, the specimens were rinsed with distilled water for 10 s and gently air dried prior to erosion.

### *Erosive Challenge and Measurement of Calcium Loss*

For determination of erosive calcium loss, the specimens (n = 10 per group) were eroded with HCl (pH 2.3, 5.01 mmol/l) for 60 s after the respective brushing treatment. Each specimen was eroded in 1 ml HCl in an Eppendorf tube, which was gently shaken during sample incubation (60×/min). The amount of calcium dissolved from the enamel and dentin surfaces by erosive treatment was analysed by atomic absorption spectroscopy (Model 2380, Perkin-Elmer, Norwalk, Conn., USA) at 422.7 nm.

**Table 1.** Percentage of calcium release (mean percentage of control, 95% CI) in the enamel groups

Group	Number of brushing strokes					
	0	10	20	50	100	500
TiF <sub>4</sub>	61.7 (50.8, 72.5) <sup>a</sup>	69.0 (60.8, 77.3) <sup>a, b</sup>	61.6 (55.9, 67.2) <sup>a</sup>	67.9 (59.7, 76.0) <sup>a</sup>	66.2 (59.1, 73.3) <sup>a</sup>	69.9 (61.4, 78.4) <sup>a</sup>
AmF	58.1 (49.7, 66.4) <sup>a</sup>	54.3 (44.7, 63.8) <sup>a</sup>	51.5 (40.7, 62.4) <sup>a</sup>	51.6 (47.5, 55.7) <sup>b</sup>	58.8 (44.5, 73.1) <sup>a</sup>	70.1 (63.3, 76.9) <sup>a</sup>
SnF <sub>2</sub>	67.3 (56.9, 77.8) <sup>A, a</sup>	74.3 (64.7, 83.9) <sup>A, B, b</sup>	77.0 (68.2, 85.8) <sup>A, B, b</sup>	77.9 (69.4, 86.4) <sup>A, B, a</sup>	85.1 (79.7, 90.6) <sup>B, b</sup>	87.9 (77.6, 98.2) <sup>B, b, *</sup>

Within each row, groups that are significantly different from each other are marked by different capital letters. Within each column, groups that are significantly different are marked by different small letters. Groups marked by an asterisk were not significantly different from the respective control.

#### *X-Ray EDS and SEM*

The surface composition of enamel and dentin specimens was obtained by X-ray EDS and SEM (SUPRA 50VP and Genesis, Carl Zeiss NTS GmbH, Oberkochen, Germany) directly after pretreatment with the test solutions and after 500 brushing strokes. Therefore, the specimens were desiccated for 4 weeks in blue silica gel [Schmidlin et al., 2001, 2002] in a vacuum evaporator. For EDS measurement a defined area of 200 × 200 μm was measured in secondary electron mode (15 kV, 100 s) with a penetration depth of approximately 3 μm. The weight percentage of the elements was analysed stoichiometrically.

For SEM examination specimens were desiccated as described above, sputter-coated with gold for 60 s and then examined at 10–20 kV.

#### *Statistical Analysis*

Calcium loss in the enamel and dentin test groups was calculated as percentage of the mean calcium loss in the respective control group (rinsed with water, brushed with 0–500 brushing strokes). Normal distribution was checked with Kolmogorov-Smirnov and Shapiro-Wilk tests. As normal distribution was found, the data were analysed by three-way ANOVA considering the kind of dental hard tissue, the number of brushing strokes or the test solution(s) as independent variable. Due to significant interaction, three-way ANOVA was followed by two-way ANOVA separately for enamel and dentin specimens [Neter et al., 1996]. Within each test group, one-way ANOVA followed by Scheffé's post-hoc test was applied to determine whether the amount of calcium released by enamel or dentin specimens, respectively, increased with increasing brushing strokes.

Differences between test and control specimens within groups brushed with the same amount of brushing strokes were analysed by one-way ANOVA followed by Scheffé's post hoc tests separately for enamel and dentin specimens.

Differences in the protective potential of the test solutions in enamel and dentin were analysed by unpaired means comparison.

The surface composition of the specimens before and after brushing (500 brushing strokes) was analysed by repeated measures ANOVA with Greenhouse-Geisser correction and independent samples tests after normal distribution (Kolmogorov-Smirnov test) were found in all groups.

SPSS (version 16, IBM, Switzerland) was used for statistical analysis. The overall level of significance was set at  $p \leq 0.05$ .

## **Results**

### *Calcium Loss*

Three-way ANOVA found the kind of dental hard tissue, the number of brushing strokes and the test solutions to be significant with respect to calcium loss. The interaction between all criteria was significant. Two-way ANOVAs showed both factors and the interaction to be significant with respect to dentin loss, while for enamel loss all factors but not the interaction were significant.

Erosive calcium losses (mean percentage of control, 95% CI) of enamel and dentin specimens are presented in tables 1 and 2. The mean calcium loss of the control groups brushed with 0–500 brushing strokes varied between 27.5 and 33.1 nmol/mm<sup>2</sup> (enamel) and between 22.9 and 25.9 nmol/mm<sup>2</sup> (dentin) and, thus, was stable over time.

Calcium release of enamel specimens which were not brushed prior to erosion was significantly reduced by AmF, TiF<sub>4</sub> and SnF<sub>2</sub> to 58–67% of control. Brushing treatment reduced the protective effect of the test solutions, but the effect was significant for SnF<sub>2</sub> only. After 500 brushing strokes, the protective effect against enamel erosion was still significant for AmF and TiF<sub>4</sub>.

In dentin, the test solutions reduced calcium release of unbrushed samples significantly to 23–31% of control. In all groups, the protective effect of the test solutions decreased with increasing brushing strokes. Application of 500 brushing strokes increased calcium release to 45–78% of control. However, the erosion-protective effect was still significant in all groups.

Generally, the protective effect of the test solutions was significantly higher in dentin compared to enamel specimens, except for AmF at 100 and 500 brushing strokes. While brushing treatment affected the protective potential of the test solutions to various extents, this effect was generally higher in dentin than in enamel.

**Table 2.** Percentage of calcium release (mean percentage of control, 95% CI) in the dentin groups

Group	Number of brushing strokes					
	0	10	20	50	100	500
TiF <sub>4</sub>	31.0 (25.2, 36.7) <sup>A, a</sup>	31.5 (27.1, 35.8) <sup>A, a</sup>	32.6 (29.5, 35.8) <sup>A, a</sup>	32.1 (28.6, 35.7) <sup>A, a</sup>	34.0 (29.4, 38.6) <sup>A, a</sup>	44.6 (38.5, 50.7) <sup>B, a</sup>
AmF	23.3 (21.3, 25.3) <sup>A, a</sup>	27.8 (24.6, 31.1) <sup>A, a, b</sup>	33.2 (27.0, 39.4) <sup>A, a</sup>	28.8 (23.9, 33.7) <sup>A, a</sup>	53.5 (45.6, 61.3) <sup>B, b</sup>	77.6 (62.1, 93.1) <sup>C, b</sup>
SnF <sub>2</sub>	23.8 (18.4, 29.2) <sup>A, a</sup>	23.7 (18.0, 29.3) <sup>A, b</sup>	24.7 (18.2, 31.2) <sup>A, a</sup>	31.3 (24.8, 37.8) <sup>A, B, a</sup>	46.7 (36.7, 56.8) <sup>B, b</sup>	62.6 (54.1, 71.1) <sup>C, b</sup>

Within each row, groups that are significantly different from each other are marked by different capital letters. Within each column, groups that are significantly different are marked by different small letters.

**Table 3.** Percentages of elements (mean ± standard deviation) on enamel surfaces after application of the test solutions (initial) and after brushing with 500 brushing strokes (BS)

Group	Time point	Ca	P	O	F	Ti	Sn	Si
Control	initial	36.9±2.2	20.3±0.5	42.2±1.9				
	after 500 BS	37.3±2.0	20.4±0.4	41.8±2.5				0.5±0.1
TiF <sub>4</sub>	initial	33.7±1.0	19.4±0.2	44.0±1.4	0.2±0.3	2.5±1.0		
	after 500 BS	33.7±1.8	19.8±0.3	44.1±2.2	0.2±0.2	1.7±0.1		0.6±0.1
AmF	initial	35.9±2.3	18.5±0.6	24.3±2.0	21.3±4.0			
	after 500 BS	36.9±1.6	19.2±2.4	37.2±2.6*	5.1±2.4*			0.5±0.1
SnF <sub>2</sub>	initial	38.2±2.4	20.3±0.5	38.9±2.0	0.1±0.1		1.9±0.3	
	after 500 BS	37.3±2.3	20.5±0.5	40.5±2.9	0.1±0.1		1.2±0.3	0.5±0.1

In elements marked by an asterisk the concentration was significantly changed by brushing compared to the initial concentration. The detection of silicium on brushed surfaces probably corresponds to remnants of the toothpaste abrasives.

### X-Ray EDS

Element composition in the control specimens was not affected by brushing.

In enamel, brushing significantly reduced the surface fluoride concentration of specimens treated with AmF. In specimens treated with SnF<sub>2</sub> and TiF<sub>4</sub> the concentration of tin or titanium, respectively, was also reduced, but this effect was not significant (table 3).

In dentin specimens, brushing reduced the surface fluoride concentration of all test groups. Also, the surface concentration of tin and titanium in specimens treated with SnF<sub>2</sub> or TiF<sub>4</sub>, respectively, was significantly reduced by brushing (table 4).

### SEM

The application of TiF<sub>4</sub> and AmF solutions resulted in the distinct formation of globular surface precipitates on enamel and dentin, while SnF<sub>2</sub> application resulted in a structurally modified surface without visible precipita-

tion on the surface (fig. 1, 2). Brushing clearly removed the surface precipitates formed after TiF<sub>4</sub> and AmF application and altered the surface treated with SnF<sub>2</sub> in a way that enamel and dentin surfaces appeared quite similar after brushing treatment.

### Discussion

This in vitro study showed that the erosion-protective effect of AmF, TiF<sub>4</sub> and SnF<sub>2</sub> was reduced by brushing over time, dentin being significantly more affected by abrasion than enamel. As brushing decreased the erosion-protective potential significantly in most groups, the null hypothesis was rejected.

The present experiment was set up as a screening procedure to evaluate to which extent the erosion-protective effect of the test solutions is affected by abrasion rather than as an attempt to reflect clinical conditions. All test

**Table 4.** Percentages of elements (mean  $\pm$  standard deviation) on dentin surfaces after application of the test solutions (initial) and after brushing with 500 brushing strokes (BS)

Group	Time point	Ca	P	O	F	Ti	Sn	Si
Control	initial	34.6 $\pm$ 2.1	18.8 $\pm$ 0.7	46.5 $\pm$ 2.7				
	after 500 BS	34.7 $\pm$ 2.1	18.3 $\pm$ 0.9	46.5 $\pm$ 2.8				0.5 $\pm$ 0.2
TiF <sub>4</sub>	initial	24.2 $\pm$ 4.8	15.7 $\pm$ 1.6	51.2 $\pm$ 3.1	2.7 $\pm$ 0.9	6.3 $\pm$ 2.5		
	after 500 BS	29.6 $\pm$ 1.9*	16.1 $\pm$ 1.2	49.9 $\pm$ 1.7	1.1 $\pm$ 0.6*	1.2 $\pm$ 0.6*		2.1 $\pm$ 1.2
AmF	initial	34.1 $\pm$ 0.9	14.7 $\pm$ 1.6	25.3 $\pm$ 1.3	26.1 $\pm$ 1.7			
	after 500 BS	33.2 $\pm$ 0.7*	17.8 $\pm$ 0.6	48.3 $\pm$ 0.9*	0.3 $\pm$ 0.3*			0.5 $\pm$ 0.2
SnF <sub>2</sub>	initial	31.4 $\pm$ 1.8	17.0 $\pm$ 1.2	43.6 $\pm$ 0.5	1.3 $\pm$ 0.5		6.8 $\pm$ 3.0	
	after 500 BS	33.9 $\pm$ 1.3	17.9 $\pm$ 0.7	44.8 $\pm$ 2.1	0.7 $\pm$ 0.3*		2.3 $\pm$ 0.6*	0.5 $\pm$ 0.2

In elements marked by an asterisk the concentration was significantly changed by brushing compared to the initial concentration. The detection of silicium on brushed surfaces probably corresponds to remnants of the toothpaste abrasives.

solutions were used at their native pH, as they were shown to be most effective (higher surface fluoride concentration and better incorporation of metal ions) at their native instead of buffered pH [Yu et al., 2010]. However, it has to be taken into consideration that the low pH of the TiF<sub>4</sub> and SnF<sub>2</sub> solutions might induce soft tissue irritations, so that the use of the test solutions as an oral mouthrinse has to be questioned.

Under clinical conditions, the presence of saliva and salivary pellicle influences the dissolution and abrasion behaviour of enamel and dentin [Hall et al., 1999; Joiner et al., 2008] as well as the formation and stability of fluoride precipitates [Ganss et al., 2007; Wiegand et al., 2008a]. However, the results of a previous *in vitro* study indicate that the resistance of CaF<sub>2</sub> precipitates against brushing abrasion is rather influenced by the presence of a pellicle. Thereby, the stability of CaF<sub>2</sub> precipitates against brushing treatment was not significantly different between specimens pretreated with artificial and human saliva [Wegehaupt et al., 2009]. Moreover, the use of a pellicle layer created *in vitro* by immersion in human saliva did not seem reasonable, as the components of human saliva are rapidly altered or degraded *in vitro* and might not reflect the bioadhesion process occurring *in vivo* [Hannig and Hannig, 2009].

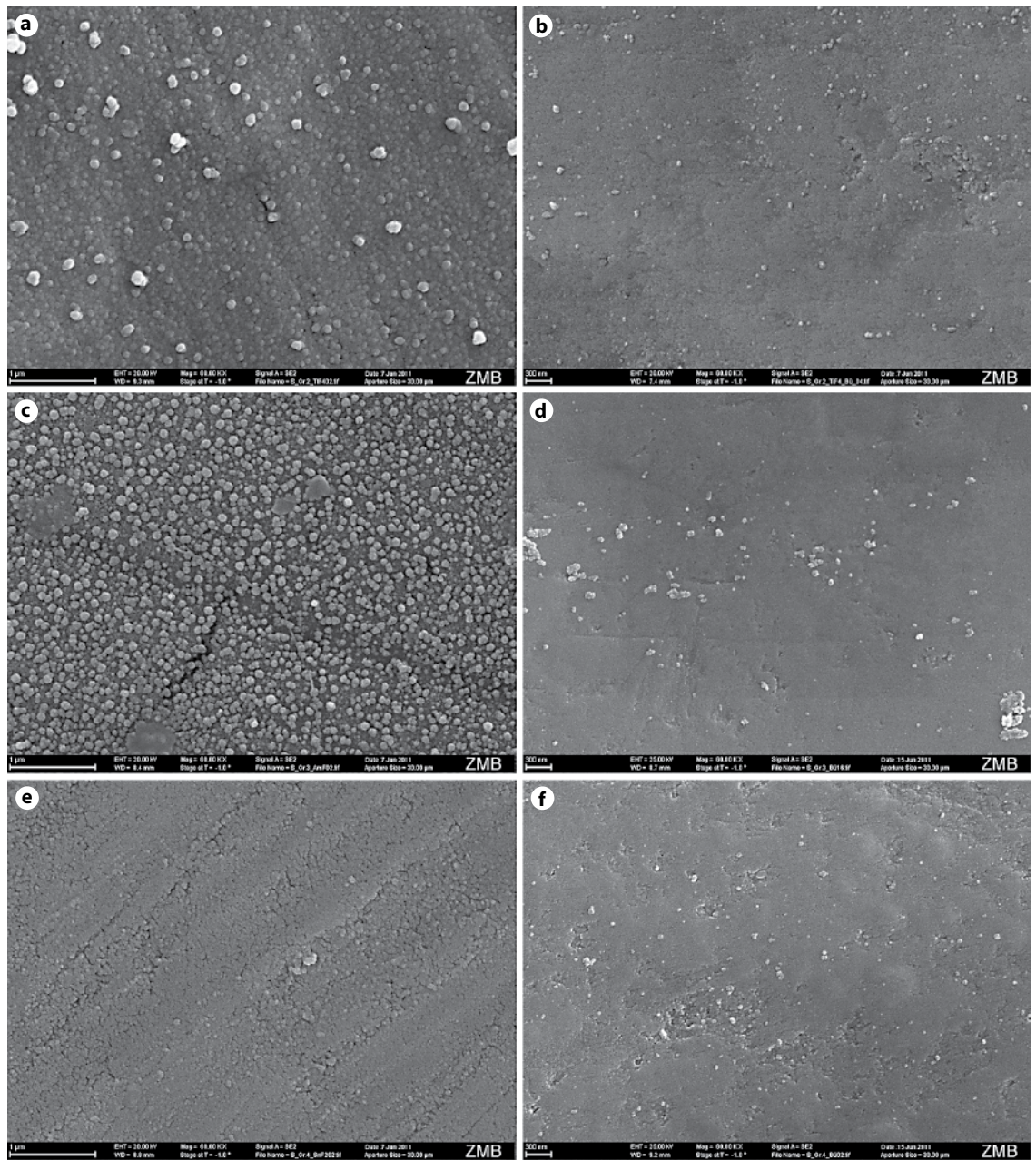
Specimens were brushed in an automatic brushing machine which ensured a standardized movement at a defined brushing force (2 N) [Wiegand and Attin, 2011]. Brushing was performed with a fluoride-free toothpaste slurry reflecting the abrasivity of common toothpastes to avoid additional fluoridation of the samples [Wegehaupt et al., 2009]. However, as oral hygiene is usually performed with fluoridated toothpastes, recharging of the

surface with fluoride might probably decrease the effect seen in the present study.

The number of brushing strokes applied was exaggerated to a total of 500 to analyse the long-term physical resistance of surface precipitates and coatings. While the application of 500 brushing strokes significantly decreased the erosion-protective potential in most groups, the application of 10–20 brushing strokes – which is equivalent to the number of brushing strokes each tooth might receive during *in vivo* toothbrushing – did not significantly reduce the efficacy of the test solutions. This observation is in accordance with a previous *in situ* study showing that the erosion-protective effect of TiF<sub>4</sub> and AmF on enamel and dentin was only slightly reduced by additional brushing (30 s twice daily) of the samples in a 3-day *in situ* erosion experiment [Wiegand et al., 2010a]. Moreover, the erosive calcium loss of specimens treated with the AmF solution correspond to the results of Attin et al. [2001] and Wegehaupt et al. [2009], where the stability of KOH-soluble fluoride against brushing was tested. Brushing with 25–100 strokes at 2.5 or 4 N brushing force, respectively, decreased the amount of KOH-soluble fluoride formed after application of sodium or amine fluoride (1% F) by 10–25%.

In the present study, the test solutions were almost equally effective in preventing enamel and dentin erosion initially, but showed slight differences at the end of the experiment. Depending on the time and frequency of application, fluoride compounds containing polyvalent metal cations were often shown to have a higher protective potential on erosion, especially when applied in cyclic erosion models without abrasion [Schlueter et al., 2009a, 2009b; Ganss et al., 2010]. In this case, large amounts of





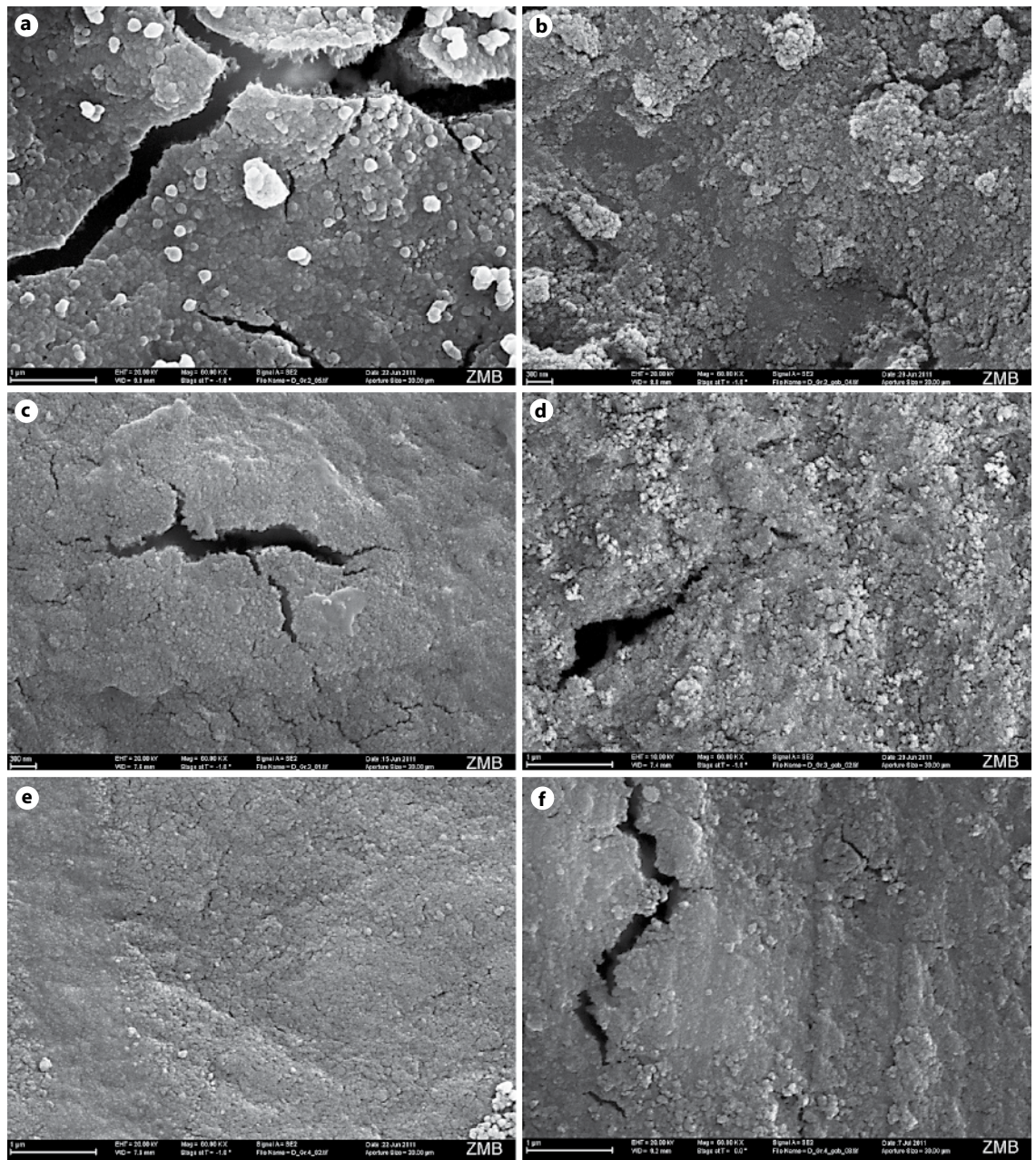
**Fig. 1.** Representative SEM images (60,000× magnification) of enamel surfaces treated with  $\text{TiF}_4$  (a, b),  $\text{AmF}$  (c, d) and  $\text{SnF}_2$  (e, f) after application of the test solutions (a, c, e) and after brushing with 500 strokes (b, d, f).

the cation are incorporated in the surface, leading to a broad structurally modified and acid-resistant zone [Schlueter et al., 2009b; Ganss et al., 2010]. As the test solutions were applied only once in the present experiment, the surface precipitates and structural alterations are limited to the outermost enamel or dentin surface, respectively. It can therefore be assumed that the abrasion stability of dental hard tissues would be increased if the test

solutions – at least those containing polyvalent cations – were frequently applied.

Remarkably, the test solutions were more effective in dentin than in enamel, but fluoridated dentin showed a higher susceptibility to abrasion compared to enamel. Laboratory experiments have often revealed a higher protective efficacy of fluoride compounds on dentin than on enamel, while in situ studies have shown the opposite.





**Fig. 2.** Representative SEM images (60,000× magnification) of dentin surfaces treated with  $\text{TiF}_4$  (a, b),  $\text{AmF}$  (c, d) and  $\text{SnF}_2$  (e, f) after application of the test solutions (a, c, e) and after brushing with 500 strokes (b, d, f).

Clinically, fluorides might be more effective in enamel than in dentin, as the organic matrix influencing the efficacy of fluorides might to some extent be affected by chemical and enzymatic degradation [Magalhaes et al., 2011], which was not simulated in the present study.

One explanation for the observation that the surface precipitates were less resistant on dentin than on enamel is the presence of the smear layer on dentin specimens,

which was not removed prior to application of the test solutions. Buchalla et al. [2007] have shown that the presence of a smear layer on dentin surfaces does not hamper fluoride penetration into dentin, but rather enhances fluoride uptake. From the results of the present study one can speculate that at least parts of the precipitates are loosely bound to the dentin surface and can be easily removed by brushing.

While the reaction of the fluoride ion with dentin has been intensively studied [Ganss et al., 2004b, 2007; Bartlett et al., 2008], little is known about how metal ions, like tin or titanium, interact with the different dentin components, especially with the organic matrix [Ganss et al., 2010]. Thus, further research is necessary to investigate the binding mechanisms of metal-containing fluoride compounds with dentin. However, although the binding mechanism of the test compounds and the adherence of the precipitates to the surface might be different, the test solutions were almost equally effective.

Within the limitations of this *in vitro* study, it can be concluded that the test solutions (AmF, TiF<sub>4</sub>, SnF<sub>2</sub>) were able to prevent erosive calcium loss, while they were generally more effective on dentin than on enamel. Brushing prior to erosion decreased the protective efficacy of the test solutions by removing the surface precipitates, but

the brushing effect was more pronounced on dentin compared to enamel. Considering a short brushing time with a small number of brushing strokes, the protective effect of the test solutions is only slightly affected.

### Author Contributions

A. Wiegand conceived and designed the experiments; S. Schneider and B. Sener performed the experiments; M. Roos and A. Wiegand analysed the data; A. Wiegand and T. Attin wrote the paper.

### Disclosure Statement

None of the authors of the present paper has a conflict of interest.

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