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Protective Effect of Different Tetrafluorides on Erosion of Pellicle-Free and Pellicle-Covered Enamel and Dentine

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Key Words
Dentine · Enamel · Erosion · Pellicle · Tetrafluoride

Abstract
The aim was to analyze the protective effects of titanium, zirconium and hafnium tetrafluorides on erosion of pellicle-free and pellicle-covered enamel and dentine in vitro. Eight groups of 20 specimens each of bovine enamel and bovine dentine were prepared. Half the specimens in each group were immersed in human saliva for 2 h for pellicle formation. Specimens were then left untreated (controls) or were treated for 120 s with TiF_4, ZrF_4 or HfF_4 solutions (0.4 or 1%) or 1.25% AmF/NaF gel. All specimens were eroded by exposure to hydrochloric acid, pH 2.6, for 25 min. Cumulative calcium release into the acid was monitored in consecutive 30-second intervals for 5 min, then at 2-min intervals up to a total erosion time of 25 min using the Arsenazo III procedure. Data were analyzed by ANOVA. 1% TiF_4 solution offered the best protective effect, especially in dentine (reduction of calcium loss about 50% at 25 min). 1% ZrF_4, 1% HfF_4 and 0.4% TiF_4 also reduced calcium loss, but to a lesser extent. Long-term effects were limited to dentine, while reduction of enamel erosion (about 25%) was restricted to 1-min erosion. The fluoride gel had a protective effect only in dentine. The efficacy of the tetrafluorides was influenced by the presence of the pellicle layer, in that the protection against dentine erosion by TiF_4 and ZrF_4 was greater on pellicle-covered specimens. Tetrafluoride solutions, especially 1% TiF_4, could decrease dental erosion, but were more effective on dentine than on enamel.

Titanium tetrafluoride (TiF_4) has been identified as a potential agent for the protection of enamel erosion, and it has been shown that 1–4% TiF_4 solutions or gels reduce mineral release and erosion depth of enamel. TiF_4 was mostly found to be more effective in inhibiting enamel erosion than sodium, stannous or amine fluoride [Tezel et al., 2002; van Rijkom et al., 2003; Hove et al., 2006; Schlueter et al., 2007]. The protective action of TiF_4 is attributed to the formation of an acid-stable surface layer, which provides mechanical protection of the surface, and to an increased fluoride uptake, which might reduce enamel demineralization chemically. In a previous study, it was shown that not only TiF_4 but also zirconium tetrafluoride (ZrF_4) and hafnium tetrafluoride (HfF_4) reduce mineral loss during short-time enamel erosion [Wiegand et al., 2008]. Mühlemann et al. [1957] and Shrestha et al. [1972] previously showed that ZrF_4 and HfF_4 reduced artificial caries lesion formation in enamel. This was attributed to both mechanical protection by formation of an amorphous coating on the enamel surface [Clarkson et

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al., 1984a, b] and increased fluoride retention [Grøn, 1977].

Less information about the effects of tetrafluorides on dentine erosion than on enamel erosion is available. Schlueter et al. [2007] demonstrated that TiF_4 was as effective as sodium fluoride in reducing the progression of erosive lesions in dentine. Application of TiF_4 to dentine specimens resulted in the formation of an electron-dense, acid-stable Ti-containing coating [Skartveit et al., 1991] as well as in increased retention of fluoride and titanium [Tveit et al., 1988; Skartveit et al., 1989a, b]. Furthermore, TiF_4 treatment was shown to be effective in decreasing dentine permeability since it probably modifies the smear layer of dentine discs to a dense and acid-resistant coating [Kazemi et al., 1999]. The reduction of dentine hypersensitivity after application of TiF_4 is also explained by the coating of titanium compounds and the increased fluoride content [Charvat et al., 1995]. As TiF_4 showed a relatively long-lasting inhibitory effect on enamel erosion [van Rijkom et al., 2003; Schlueter et al., 2007], it seemed interesting to evaluate and compare the long-term effects of tetrafluorides on dentine.

Furthermore, it seemed worth investigating whether lower tetrafluoride concentrations (≤1%) are sufficient to reduce dental erosion. Vieira et al. [2005] found no difference in the erosion-inhibiting effect of 1 and 4% TiF_4 gels. Also, a previous study on the impact of different tetrafluoride compounds on short-term enamel erosion revealed no differences between 4 and 10% tetrafluoride solutions [Wiegand et al., 2008]. Thus, it might be speculated that lower doses of tetrafluoride might also inhibit enamel and dentine erosion.

Most studies of the impact of tetrafluorides on dental erosion were done without the presence of an acquired salivary pellicle on the specimens [van Rijkom et al., 2003; Vieira et al., 2005; Hove et al., 2006; Schlueter et al., 2007]. The protein-binding properties of titanium [Mundorff et al., 1972; Gu et al., 1996] suggest that the effect of TiF_4 might be influenced by the organic pellicle layer. Hove et al. [2007] showed that the protective effect of TiF_4 on erosion of bovine enamel was increased in the presence of an in vitro salivary pellicle. Currently, information about the efficacy of ZrF_4 and HfF_4 applied on pellicle-coated dental hard tissues is missing.

In view of the above considerations, the present study aimed to evaluate the effects of 0.4 and 1% TiF_4, ZrF_4 and HfF_4 on the erosion of pellicle-free and pellicle-covered enamel and dentine specimens. The effects of the tetrafluorides were compared with that of a commercially available 1.25% fluoride gel.

Table 1. pH and fluoride content of the test agents

Test agent	pH	Concentration, mM	Fluoride concentration	
			wt %	mM
Elmex gelée	4.9	NA	1.25	660
TiF_4 1%	1.3	80	0.61	323
TiF_4 0.4%	1.7	32	0.25	135
ZrF_4 1%	2.1	60	0.45	239
ZrF_4 0.4%	2.4	24	0.18	95
HfF_4 1%	2.1	40	0.30	159
HfF_4 0.4%	2.3	16	0.12	64

NA = Not available.

Materials and Methods

Preparation of Enamel and Dentine Specimens

Enamel and dentine specimens (3 mm in diameter, n = 160) were prepared from freshly extracted, undamaged bovine incisors and embedded in acrylic resin blocks 6 mm in diameter (Paladur, Heraeus Kulzer, Hanau, Germany). To prepare dentine cylinders, enamel was completely removed until dentine was just exposed. The labial surfaces of the specimens were ground flat and polished with water-cooled carborundum discs (500–4,000 grit, waterproof silicon carbide paper, Stuers, Erkrath, Germany) thereby removing approximately 200 μm of the outermost layer as checked with a micrometer (Digimatic, Mitutoyo, Tokyo, Japan). The enamel and dentine specimens were distributed among eight groups of 20.

Salivary Treatment

Prior to the experiment, 10 enamel or dentine specimens of each group were exposed in vitro to pooled human saliva for 120 min. Each specimen was immersed in 0.5 ml freshly collected pooled saliva without agitation. Unstimulated human saliva was collected between 8 and 8.30 a.m. from 2 male and 3 female healthy volunteers aged between 21 and 60 years. The volunteers had no active carious lesions, erosions or salivary dysfunction and did not use any kind of medication. Two hours before and during the collection of saliva the subjects were advised not to eat or drink.

Fluoride Agents

The following tetrafluoride solutions were used: 1% TiF_4, 0.4% TiF_4, 1% ZrF_4, 0.4% ZrF_4, 1% HfF_4 and 0.4% HfF_4 (Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany) in distilled water. The solutions were remixed for 120 s on an orbital shaker (Vortex, Schütt Labortechnik, Germany) prior to application; 25 μl of solution was applied to each specimen surface and left undisturbed for 120 s. Groups of 20 enamel and dentine specimens were treated with a fluoride gel (1.25% F, Elmex gelée, GABA, Lörrach, Germany, lot 849703) in the same way. After treatment, specimens were rinsed with distilled water for 15 s. Enamel and dentine specimens (20 of each: 10 pellicle-free, 10 pellicle-covered) were not treated with any test agent and served as controls. The pH and fluoride concentration of the test agents are given in table 1.

Erosion Experiment

Each specimen was fixed in a brass jig, which allowed exposure of the specimen surfaces to a small erosion chamber of 1-µl volume (2 mm in diameter × 0.3 mm in height). Hydrochloric acid (2.5 mM, pH 2.6) was pumped at 60 µl/min from a reservoir outside the chamber into the space erosion chamber (fig. 1). Prior to acid exposure, distilled water was rinsed through the chamber to ensure bubble-free flow. Because of the small height of the space, bubbles did not form during flow of the acid. Six chambers were each connected to a multichannel pump (Ismatec, Glattbrugg, Switzerland). Volume measurement of 36 3-min fractions showed that the coefficient of variation of the acid flow rate was 2.6%.

Samples were exposed to acid for a total of 25 min. For the first 5 min the acid was collected at consecutive 30-second intervals via an outlet pipe into a reservoir. The acid was then collected at consecutive 2-min intervals up to a total erosion time of 25 min. The acid collected during the successive intervals was analyzed for calcium.

Calcium Analysis

Erosive demineralization was assessed by colorimetric analysis of calcium release into the acid, using the Arsenazo III method (Fluitest, Ca-A-II, Analyticon, Lichtenfels, Germany) [Attin et al., 2005] on 10-µl samples in microtiter plates. The lowest standard of the calibration curve (0.4 nmol Ca/10 µl acid) was considered as a threshold for the detection limit of the procedure. In a preliminary test, the calcium release from pellicle-covered enamel specimens (n = 4) into distilled water and pellicle-covered acrylic resin (Paladur, Heraeus Kulzer) specimens (n = 6) into HCl was measured to analyze whether pellicle itself provides a reservoir of calcium ions. For both conditions, the calcium released was below the detection limit.

Statistical Analysis

Cumulative calcium release in each interval was calculated. Data were statistically analyzed by three-way ANOVA, with 'kind of dental hard tissue' (two levels: enamel and dentine), the 'test agent' (eight levels: 0.4 and 1% TiF_4, ZrF_4 or HfF_4 solutions, 1.25% fluoride gel and control) and 'salivary pretreatment' (two levels: pellicle-free and pellicle-covered specimens) as factors. ANOVA was followed by Dunnett and Bonferroni/Dunn post-hoc tests. At 1, 5, 15 and 25 min mean differences in calcium loss between the test groups and the respective controls together with the 95% confidence intervals were computed and analyzed by two-sample analysis. Within each group, mean differences in calcium loss of pellicle-free and pellicle-covered specimens at t = 1, 5, 15 and 25 min also analyzed by two-sample analysis. The level of significance was set at $p < 0.05$.

Results

The mean rate of calcium release (µmol/cm^2 · min) from pellicle-free enamel was stable over time, amounting to 0.41 at 1 min, 0.43 at 5 min, 0.44 at 15 min and 0.45 at 25 min. For dentine, the mean calcium release rate (µmol/cm^2 · min) of the pellicle-free controls decreased over time (0.45 at 1 min, 0.41 at 5 min, 0.34 at 15 min and 0.30 at 25 min). Relative cumulative calcium losses (mean % of control) of enamel and dentine in the different groups are presented in figure 2.

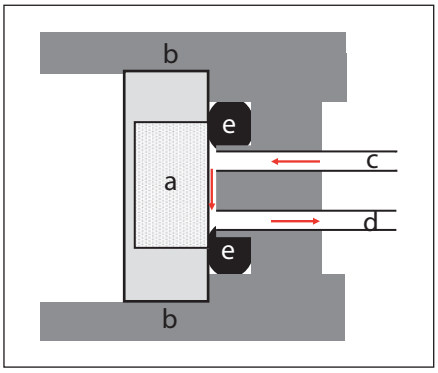


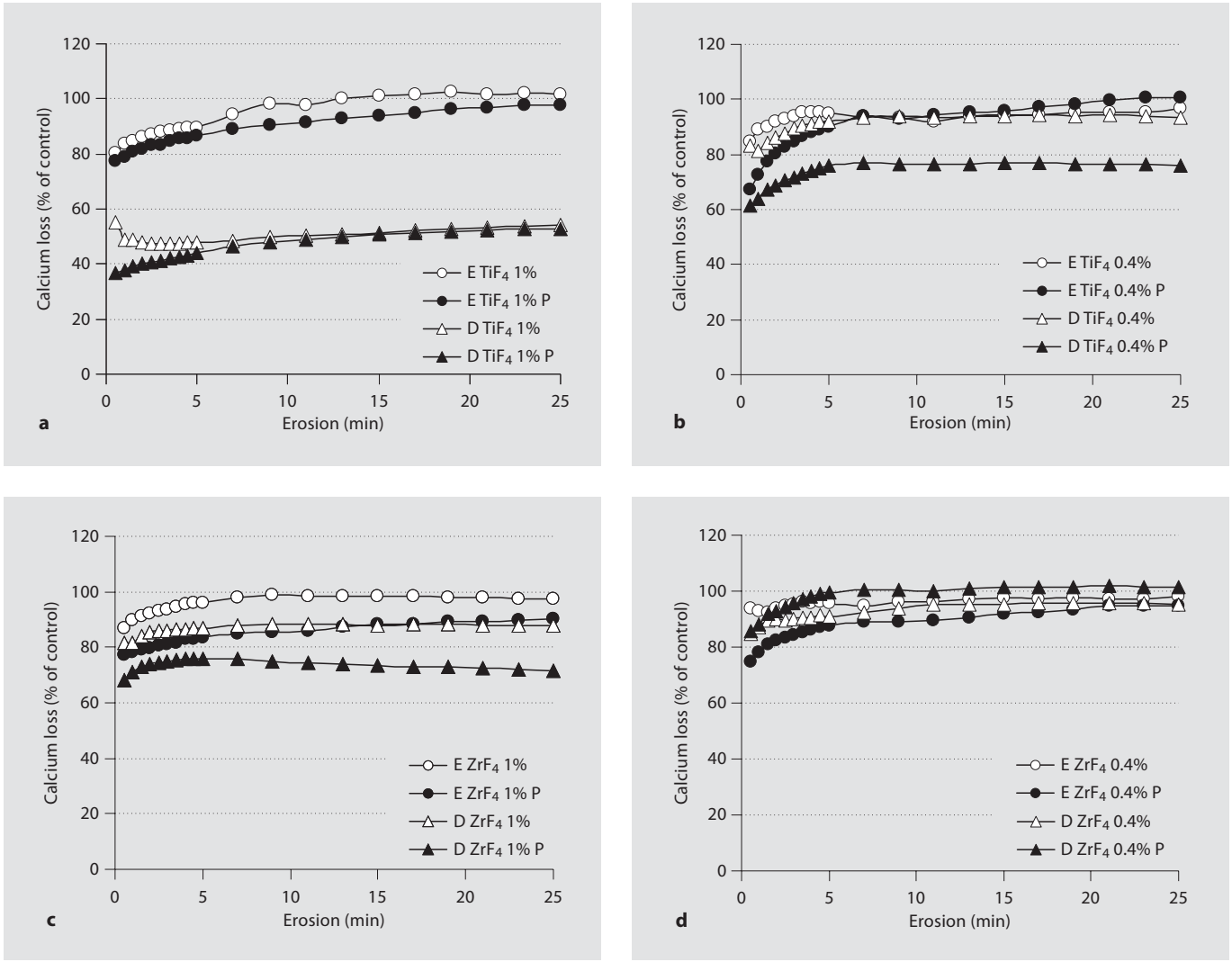
Fig. 1. Design of the erosion chamber. The enamel or dentine specimen (a, 3 mm in diameter) was fixed in a metal jig (b). Hydrochloric acid was pumped through an inlet tube (c, inner diameter 0.45 mm) over the specimen surface (size of contact area: diameter 2 mm, height 0.3 mm) and collected via an outlet tube (d, inner diameter 0.45 mm). A rubber O-ring (e) ensured leak tightness.

Mean differences (test – control) in calcium loss (µmol/cm^2) among the test groups and the pellicle-free and pellicle-covered controls at 1, 5, 15 and 25 min are presented in table 2 for enamel and table 3 for dentine.

Three-way ANOVA found the main factors 'kind of dental hard tissue', 'test agent' and 'saliva pretreatment' to be significant at 1, 5, 15 and 15 min. At all time points, the factors 'kind of dental hard tissue' and 'test agent' showed significant interactions. Post-hoc tests revealed significant differences between enamel and dentine specimens, between the different test groups and pellicle-covered and pellicle-free specimens at all time points.

Two-sample analysis revealed that 0.4 and 1% TiF_4, 1% ZrF_4 and 1% HfF_4 solutions as well as the 1.25% fluoride gel decreased calcium release from dentine up to 25 min (table 3). The greatest effect was observed after application of 1% TiF_4 (fig. 2a), which showed significantly better dentine protection (calcium release: 38–49% of control) than 0.4% TiF_4, 1% ZrF_4 and 1% HfF_4. The fluoride gel offered a significant protective effect on dentine erosion during the whole observation period, but mean calcium loss increased from 27–34% at 1 min to 74–84% at 25 min.

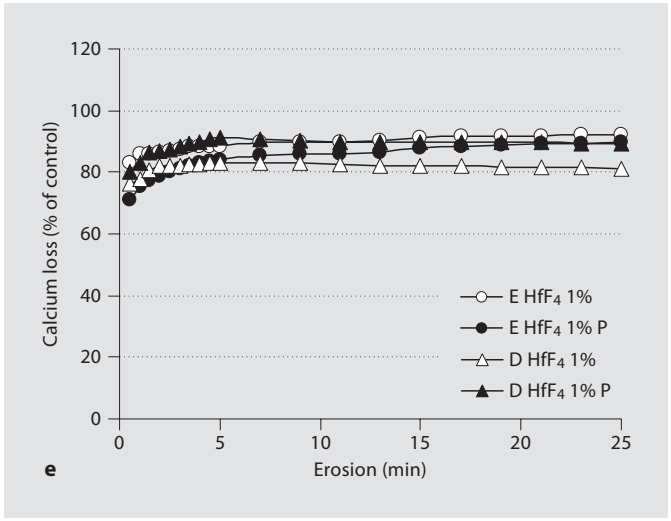
For enamel, significant protective effects of the tetrafluorides (except 0.4% HfF_4) were limited to 1 min. The fluoride gel did not show any protective effect on enamel (table 2).

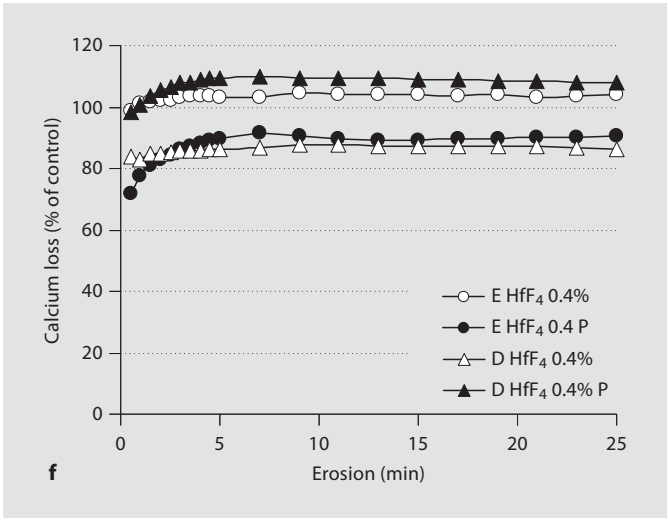


Two-sample analysis revealed significant differences between pellicle-covered and pellicle-free dentine specimens in groups treated with 0.4% TiF_4 (up to 25 min), 1% TiF_4 (up to 15 min), 1% ZrF_4 (up to 25 min) and 0.4% HfF_4 (1 min). In these groups, mean differences in calcium loss ($\mu mol/cm^2$) between pellicle-covered and pellicle-free dentine amounted to: 0.4% TiF_4 –2.597 at 25 min; 1% TiF_4 –0.504 at 15 min; 1% ZrF_4 –2.421 at 25 min, and 0.4% HfF_4 0.002 at 1 min. In enamel, calcium loss ($\mu mol/cm^2$) of pellicle-covered and pellicle-free specimens was significantly different in groups treated with 1% ZrF_4 (mean difference –0.994 at 15 min) and 0.4% HfF_4 (mean difference –2.242 at 25 min). In controls there was no significant difference between pellicle-covered and pellicle-free enamel and dentine specimens.

Discussion

The present study shows that tetrafluoride solutions can reduce dentine erosion up to 25 min, but offer only short-term protection (<1 min) from enamel erosion. Among the tetrafluoride solutions, 1% TiF_4 solution offered the best protection, while 1% ZrF_4, 1% HfF_4 and 0.4% TiF_4 were less effective. However, it has to be taken into account that the tetrafluoride solutions were not applied in equimolar concentrations. Thus, the different fluoride concentrations of the tetrafluoride solutions (table 1) might also account for the different protective capability of the agents and for the higher efficacy of 1% TiF_4.





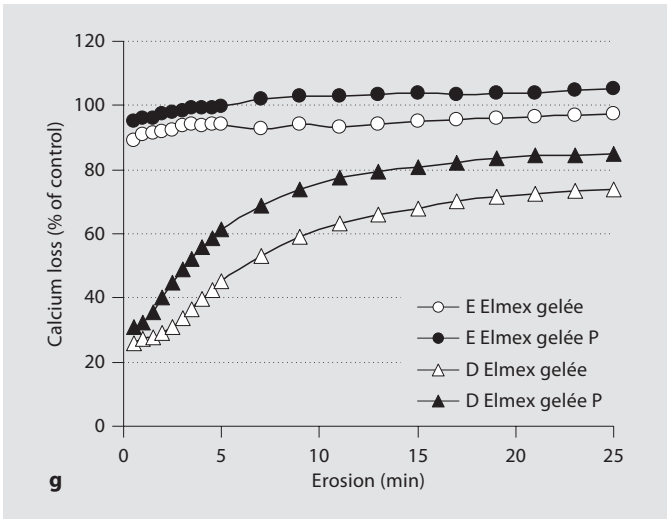


Fig. 2. Mean relative cumulative calcium losses from enamel (E) and dentine (D) (tests as percentage of respective controls). Open symbols = pellicle-free; solid symbols = pellicle-covered (P).

Erosion by hydrochloric acid, pH 2.6, is found in patients suffering from reflux or vomiting attacks, which bring gastric acid into the oral cavity. Acid exposure of up to 25 min was selected to investigate the longevity of the protective capability of the test agents. As this study focused on the longevity of the tetrafluoride effects, the erosive challenge of the specimens was not intermitted by remineralization periods. In the clinical situation, saliva exposure might counterbalance the demineralization of the specimens to a certain degree and thus influence the results. Erosion of the specimens was performed by continuous acid flow, to prevent saturation of the acid, which might take place under static conditions.

The tetrafluoride solutions were applied only once in order to simulate professional fluoride application by a dentist. For better comparison, the commercially available fluoride gel was also applied only once on the specimens. Bovine dental tissues were used as a substrate as they are widely used in erosion research and their chemical and mechanical properties are similar to human dental hard tissues. However, it should be borne in mind that the susceptibility to erosion might differ between bovine and human hard tissues [Rios et al., 2006; Attin et al., 2007]. Hove et al. [2007] found a significant difference in the protective effect of TiF_4 (0.5 M F) on human and bovine enamel; pretreatment with TiF_4 led to significantly less erosion in bovine enamel, while untreated bovine and human enamel showed the same degree of erosion. These results should be taken into account for extrapolation from in vitro data to the clinical situation. The meth-

Table 2. Mean differences between test groups and respective controls (test – control) in enamel calcium loss (μmol/cm^2) at 1, 5, 15 and 25 min

Groups	Pellicle	1 min	5 min	15 min	25 min
Elmex gelée	PF	–0.04 (–0.08, –0.05)	–0.13 (–0.41, +0.16)	–0.33 (–1.19, +0.53)	–0.28 (–1.76, +1.21)
	PC	–0.02 (–0.11, –0.03)	–0.01 (–0.34, +0.33)	+0.23 (–0.79, +1.26)	+0.54 (–1.17, +2.24)
1% TiF_4	PF	–0.07 (–0.13, –0.004)	–0.22 (–0.50, +0.06)	+0.09 (–0.76, +0.95)	+0.18 (–1.29, +1.64)
	PC	–0.09 (–0.15, –0.02)	–0.28 (–0.62, +0.06)	–0.37 (–1.44, +0.70)	–0.23 (–2.02, +1.55)
0.4% TiF_4	PF	–0.45 (–0.12, +0.03)	–0.11 (–0.45, +0.24)	–0.38 (–1.27, +0.52)	–0.38 (–1.87, +1.12)
	PC	–0.11 (–0.20, –0.02)	–0.20 (–0.61, +0.21)	–0.27 (–1.90, +0.85)	+0.08 (–1.73, +1.90)
1% ZrF_4	PF	–0.04 (–0.12, +0.03)	–0.08 (–0.39, +0.23)	–0.10 (–0.96, +0.76)	–0.27 (–1.75, +1.22)
	PC	–0.09 (–0.16, –0.02)	–0.36 (–0.68, +0.01)	–0.74 (–1.78, +0.30)	–1.01 (–2.76, +0.75)
0.4% ZrF_4	PF	–0.03 (–0.11, +0.05)	–0.09 (–0.43, +0.25)	–0.16 (–1.20, +0.88)	–0.24 (–1.90, +1.43)
	PC	–0.09 (–0.15, –0.02)	–0.26 (–0.59, +0.08)	+0.50 (–1.58, +0.57)	–0.52 (–2.34, +1.31)
1% HfF_4	PF	–0.06 (–0.12, +0.004)	–0.25 (–0.52, +0.02)	–0.58 (–1.30, +0.15)	–0.86 (–2.09, +0.36)
	PC	–0.10 (–0.17, –0.03)	–0.33 (–0.70, +0.05)	–0.76 (–1.97, +0.44)	–1.06 (–3.06, +0.95)
0.4% HfF_4	PF	+0.01 (–0.07, +0.78)	+0.07 (–0.22, +0.36)	+0.28 (–0.51, +1.07)	+0.44 (–0.78, +1.66)
	PC	–0.90 (–0.20, +0.01)	–0.22 (–0.70, +0.27)	–0.68 (–2.12, +0.77)	–0.96 (–3.32, +1.41)

95% confidence intervals in parentheses. Mean cumulative calcium loss (± SD, μmol/cm^2) of pellicle-free (PF) and pellicle-covered (PC) enamel controls, respectively, was: 0.41 ± 0.08 and 0.40 ± 0.08 at 1 min; 2.12 ± 0.33 and 2.05 ± 0.40 at 5 min; 6.57 ± 0.85 and 6.21 ± 1.21 at 15 min; 11.09 ± 1.42 and 10.25 ± 2.04 at 25 min.

Table 3. Mean differences between test groups and respective controls (test – control) in dentine calcium loss (μmol/cm^2) at 1, 5, 15 and 25 min

Groups	Pellicle	1 min	5 min	15 min	25 min
Elmex gelée	PF	–0.36 (–0.40, –0.21)	–1.19 (–1.51, –0.87)	–1.84 (–2.88, –0.80)	–2.32 (–3.99, –0.64)
	PC	–0.26 (–0.21, –0.11)	–0.69 (–0.99, –0.39)	–0.93 (–1.83, –0.03)	–1.14 (–2.63, +0.35)
1% TiF_4	PF	–0.23 (–0.31, –0.15)	–1.13 (–1.49, –0.77)	–2.80 (–3.84, –1.75)	–4.10 (–5.74, –2.46)
	PC	–0.24 (–0.29, –0.19)	–1.01 (–1.24, –0.77)	–2.39 (–3.15, –1.64)	–3.55 (–4.85, –2.25)
0.4% TiF_4	PF	–0.08 (–0.17, +0.03)	–0.18 (–0.53, +0.17)	–0.35 (–1.35, +0.64)	–0.58 (–2.17, +1.01)
	PC	–0.14 (–0.19, –0.09)	–0.43 (–0.70, –0.16)	–1.13 (–1.96, –0.30)	–1.79 (–3.21, –0.37)
1% ZrF_4	PF	–0.08 (–0.17, +0.001)	–0.28 (–0.63, +0.07)	–0.70 (–1.80, +0.41)	–1.10 (–2.95, +0.75)
	PC	–0.11 (–0.16, –0.06)	–0.43 (–0.69, –0.18)	–1.28 (–2.05, –0.52)	–2.14 (–3.42, –0.86)
0.4% ZrF_4	PF	–0.06 (–0.14, +0.03)	–0.20 (–0.51, +0.13)	–0.27 (–1.24, +0.69)	–0.41 (–2.03, +1.21)
	PC	–0.05 (–0.11, +0.01)	–0.01 (–0.31, +0.30)	+0.06 (–0.94, +1.06)	+0.12 (–1.58, +1.81)
1% HfF_4	PF	–0.10 (–0.19, –0.01)	–0.37 (–0.72, –0.02)	–1.05 (–2.02, –0.07)	–1.68 (–3.24, –0.12)
	PC	–0.07 (–0.11, –0.02)	–0.16 (–0.39, +0.06)	–0.50 (–1.17, +0.18)	–0.81 (–1.98, +0.36)
0.4% HfF_4	PF	–0.07 (–0.15, +0.02)	–0.25 (–0.57, +0.07)	–0.56 (–1.49, +0.37)	–0.93 (–2.45, +0.59)
	PC	–0.004 (–0.05, +0.04)	+0.12 (–0.10, +0.07)	+0.27 (–0.49, +1.02)	+0.30 (–1.03, +1.62)

95% confidence intervals in parentheses. Mean cumulative calcium loss (± SD, μmol/cm^2) of pellicle-free (PF) and pellicle-covered (PC) dentine controls, respectively, was: 0.45 ± 0.11 and 0.39 ± 0.06 at 1 min; 2.17 ± 0.43 and 1.79 ± 0.30 at 5 min; 5.76 ± 1.26 and 4.85 ± 0.96 at 15 min; 8.90 ± 2.02 and 7.5 ± 1.68 at 25 min.

od of calcium analysis allows quantitation of erosion using small acid volumes [Attin et al., 2005; Hannig et al., 2005]. Several previous studies also used calcium analysis to measure dissolution of TiF_4-treated specimens [van Rijkom et al., 2003; Hove et al., 2007]. Similar enamel calcium levels in consecutive acid fractions indicate that the outer layers of enamel provide equal resistance to erosion. As found previously, dentine calcium loss of untreated specimens decreased with increasing acid exposure [Hunter et al., 2000a, b], which might be explained by an increased buffering capacity of the exposed demineralized organic matrix [Ganss et al., 2004].

The commercially available fluoride gel did not reduce enamel erosion under the present in vitro conditions. Similarly, Vieira et al. [2005] found no protective effect of a 1% amine fluoride gel on enamel in a pH-cycling model (total erosion time 72 min). Previous studies indicate that the effect of amine fluoride gels might be increased by multiple applications [Lagerweij et al., 2006; Lennon et al., 2006]. As the acidic amine fluoride gel was applied only once in the present study, it might be that only a thin CaF_2-like layer was formed on enamel, and that it was not sufficient to inhibit erosion substantially.

In contrast to enamel, amine fluoride gel was effective in preventing dentine calcium loss and inhibited dentine erosion nearly completely in the initial phase of erosion. This might be explained by the buffering capacity of the organic matrix of dentine, which could reduce the pH fall at the demineralizing front. Combined with high amounts of fluoride this might result in an inhibition of demineralization [Ganss et al., 2004].

Generally, long-term effects of tetrafluoride protection were limited to dentine erosion. The protective effect of the different tetrafluoride agents on enamel was smaller than that reported by Hove et al. [2006, 2007], who observed an 88% reduction in erosion depth [Hove et al., 2006] and a reduction of 56–76% in calcium loss [Hove et al., 2007] in TiF_4-treated enamel. The different results might be partly explained by the lower fluoride concentrations of the tetrafluoride solutions (0.064–0.323 M F) used in the present study. However, unlike Hove et al. [2006, 2007], Vieira et al. [2006] and Magalhães et al. [2007] found no protective effect of a 4% TiF_4 solution on long-term enamel erosion. It was suggested that the glaze formed upon TiF_4 application did not withstand the effect of a prolonged erosive challenge.

While the protective effect of the amine fluoride gel distinctly decreased during acid exposure, the protective capacity of the 1% TiF_4 solution on dentine remained stable over time. Moreover, the tetrafluoride solutions, especially 1% TiF_4, showed a more prolonged protective effect on dentine than on enamel erosion, which might partly be attributed to the formation of an acid-stable coating. Further, the increased protective effect in dentine compared to enamel specimens might be explained by the higher content of organic components in dentine, which might play an important role in the fluoride uptake from tetrafluorides. Gu and Söremark [1996] showed that the enamel fluoride uptake by TiF_4 was distinctly reduced when the organic components were removed by NaOCl. Moreover, Mundorff et al. [1972] observed that TiF_4 failed to produce a high-order glaze on organic-reduced enamel. These findings might be extrapolated to dentine. In this context it was assumed that the titanium ion might play an essential role because of its complexing ability and protein-binding properties [Mundorff et al., 1972]. The important role of the titanium ion might explain the better protective capability of 1% TiF_4 on dentine erosion compared to 0.4% TiF_4, 1% ZrF_4 and 1% HfF_4 in the present study.

The results of the present study indicate that the protective effect of TiF_4 and ZrF_4 might be enhanced by the presence of the pellicle layer, at least on dentine specimens. Similarly, Hove et al. [2007] showed that the protective effect of TiF_4 on enamel erosion was increased in the presence of an in vitro salivary pellicle. It might be speculated whether special binding mechanisms between these tetrafluorides and the acquired pellicle might be responsible for this effect. Surprisingly, the pellicle layer did no have a significant protective effect on enamel and dentine control specimens. This might be related to the fact that an experimental in vitro formed pellicle might be too immature compared to a salivary pellicle formed in situ. Statistical analysis did not show significant effects of pellicle on the effect of the fluoride gel. Even so, from figure 2g it might be speculated that the pellicle might impede the effect of fluoride in dentine.

In conclusion, 0.4 and 1% TiF_4, 1% ZrF_4 and 1% HfF_4 solutions can decrease calcium loss from enamel and dentine during erosion, but the long-term effect is limited and restricted to dentine. It seems worth investigating whether carriers with a better capability to adhere on dental surfaces, such as gels or varnishes, might increase their protective effect. Moreover, the efficacy of tetrafluoride agents has to be confirmed in situ.

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