Comparison of the Effects of TiF₄ and NaF Solutions at pH 1.2 and 3.5 on Enamel Erosion in vitro

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Key Words
Calcium · Enamel · Erosion · Fluoride · Profilometry · Scanning electron microscopy

Abstract
This study aimed to analyse and compare the protective effect of buffered (pH 3.5) and native (pH 1.2) TiF₄ in comparison with NaF solutions on enamel erosion. Bovine samples were pretreated with 1.50% TiF₄ or 2.02% NaF (both 0.48 M F) solutions, each at a pH of 1.2 and 3.5. The control group received no fluoride pretreatment. Twenty samples per group were eroded with HCl (pH 2.6) for 10 × 60 s. Erosion was either investigated by profilometry (n = 10) or by determination of calcium release into the acid (n = 10). Additionally, the elemental surface composition was quantified by X-ray energy-dispersive spectroscopy in fluoridated but not eroded samples (6 samples per group). Scanning electron microscopy was performed prior and after erosion (2 samples per group). Cumulative enamel loss (μm) and calcium release (nmol/mm²) were analysed by repeated-measures ANOVA. The Ti and F surface composition was analysed by one-way ANOVA separately for each element. Only TiF₄ at pH 1.2 reduced enamel surface loss significantly. Calcium release was significantly reduced by TiF₄ and NaF at pH 1.2, but not by the solutions at pH 3.5. Samples pretreated with TiF₄ at pH 1.2 showed a significant increase in Ti, while NaF increased F concentration significantly. Only TiF₄ at pH 1.2 induced the formation of a glaze-like layer, which was still present after erosion. Enamel erosion can be significantly reduced by TiF₄ at pH 1.2 but not at pH 3.5. TiF₄ at pH 1.2 was more effective in protecting against enamel erosion than NaF.

Titanium tetrafluoride (TiF₄) is known to prevent not only carious but also erosive demineralisation. Several in vitro studies found TiF₄ to be equally or more effective in reducing erosive enamel and dentine lesions than NaF, AmF or SnF₂ [van Rijkom et al., 2003; Schlueter et al., 2007; Hove et al., 2007a, 2008]. While the efficacy of sodium or amine fluoride to prevent erosion is related to the formation of CaF₂ precipitates [Petzold, 2001; Wiegand and Attin, 2003; Ganss et al., 2007], the protective action of TiF₄ is mainly attributed to the formation of a glaze-like surface coating. This acid-resistant layer is assumed to be composed of titanium oxide [Wei et al., 1976; Tveit...
et al., 1988] or of organometallic complexes [Mundorff et al., 1972]. It is also assumed that the application of TiF$_4$ leads to an increased fluoride uptake, which might reduce demineralisation chemically. Increased enamel fluoride uptake after the application of TiF$_4$ might be explained by the ability of the polyvalent metal ion to form strong fluoride complexes while simultaneously binding firmly to enamel apatite crystals [McCann, 1969; Wefel and Harless, 1981, 1982].

However, TiF$_4$ solutions and varnishes present very low pH (pH 1–2) [Hove et al., 2006; Magalhães et al., 2007; Schlüeter et al., 2007]. Although this might enhance the depth of penetration by fluoride ions into enamel, the low pH is considered as a major drawback of these agents as it does not allow self-application by the patient. For potential home use of TiF$_4$, products with higher pH that are as effective as the agents with low pH would be desirable.

In a recent study by Exterkate and ten Cate [2007], the protective potential of a titanium fluoride derivative at higher pH (pH 3) on artificial enamel caries was analysed. Thereby the titanium fluoride derivative inhibited the development of caries-like enamel lesions nearly completely, while a NaF solution with pH 3 failed to reduce enamel demineralisation. As yet, there is a lack of studies analysing the effects of TiF$_4$ agents at pH higher than 1–2 on enamel erosion. In one study, TiF$_4$ gels with pH 2.5 (1% TiF$_4$) and pH 3.2 (4% TiF$_4$) failed to reduce enamel erosion by citric acid significantly [Vieira et al., 2005]. However, in that study, the TiF$_4$ gels were not applied in equimolar concentrations of fluoride. The impact of different pH values on the efficacy of equimolar solutions of TiF$_4$ to prevent dental erosion has not been investigated and compared as yet. Thus, it was the aim of the present study to analyse the effect of native (pH 1.2) and buffered (pH 3.5) TiF$_4$ solutions on enamel erosion and to compare the effects with NaF solutions of the same pH.

The null hypotheses tested were that the efficacy of TiF$_4$ at pH 1.2 and 3.5 is not significantly different and that TiF$_4$ and NaF of the same pH perform similarly, independently of pH.

**Material and Methods**

**Experimental Set-Up**

Enamel samples were pretreated with one of the respective fluoride solutions (1.50% TiF$_4$, pH 1.2 and 3.5; 2.02% NaF, pH 1.2 and 3.5, n = 20 for each treatment) and subjected to erosive treatment with hydrochloric acid (pH 2.6, 2.5 mM) for 10 × 60 s. Samples not treated with fluoride served as controls. Enamel erosion was analysed by profilometry (experiment 1; 10 samples/group) or determination of calcium release into the acid (experiment 2; 10 samples/group). Additionally, fluoridated but not eroded samples were examined by X-ray energy-dispersive spectroscopy (EDS, experiment 3; 6 samples/group). Scanning electron microscopy (SEM) was performed prior to and after erosion in 2 samples per group. The total number of enamel samples in each group was 28.

**Sample Preparation**

Enamel samples were obtained from freshly extracted, non-damaged bovine incisors, which were stored in 0.9% NaCl solution until used. For experiment 1, bovine crown specimens were used. For experiments 2 and 3, cylindrical enamel samples (3 mm in diameter) were prepared from bovine crowns with a hollow drill and embedded in acrylic resin (Paladur, Heraeus Kulzer, Germany). The labial surfaces of the cylindrical specimens and of the crowns were ground flat and polished with water-cooled boronundum discs (1,200-, 2,400- and 4,000-grit, waterproof silicon carbide paper, Stuers, Birmensdorf, Switzerland) thereby removing approximately 200 μm of the outermost layer as checked with a micrometer (Digimatic, Mitutoyo, Tokyo, Japan). Only samples without cracks or alterations on the sample surfaces were selected for the study.

The polished surfaces of the crown specimens were covered with adhesive tape (Tesa, Beiersdorf, Hamburg, Germany) leaving a window of 5 × 2 mm. The tape was stuck to the enamel surface during the fluoride and erosion treatment and ensured the maintenance of reference surfaces to measure the depth of enamel loss thereafter. Thus the area of the exposed enamel surfaces was 10 mm$^2$ (experiment 1) and 7.1 mm$^2$ (experiments 2 and 3). The samples were randomly assigned to the five groups.

**Fluoride Solutions and Treatment**

Fluoride solutions of pH 1.2 or 3.5 were prepared as follows: 1.50% TiF$_4$ (0.48 M F, 0.12 M Ti, pH 1.2) was obtained by mixing 1.5 g titanium tetrafluoride powder (Stream Chemicals, Newburyport, Mass., USA) with 100 ml ultrapure water. The 1.50% TiF$_4$ solution was adjusted to pH 3.5 by adding 2.3 g sodium citrate/100 ml. The 2.02% NaF solutions (Merck, Switzerland, 0.48 M F) were adjusted to pH 1.2 and 3.5 by adding 45 g H$_3$PO$_4$/100 ml and 12.6 g 5 M H$_3$PO$_4$/100 ml, respectively. The solutions were prepared freshly prior to application to the enamel specimens.

For fluoride pretreatment, 15 μl (experiment 1, 1.5 μl/mm$^2$) or 10 μl (experiments 2 and 3, 1.4 μl/mm$^2$) of the respective solutions (1.50% TiF$_4$, pH 1.2 and 3.5; 2.02% NaF, pH 1.2 and 3.5) were pipetted on the enamel surface and left undisturbed for 60 s. After treatment, specimens were rinsed with 50 ml distilled water for 15 s. Specimens of the control group were treated with distilled water only.

Thereafter, enamel samples were submitted to erosion with hydrochloric acid (pH 2.6, 2.5 mM) for 10 × 60 s in sequence at room temperature. Each sample was stored for 60 s in 2 ml (experiment 1) or 1 ml (experiments 2 and 3) of HCl in an Eppendorf tube, which was gently shaken (60 x/min) during sample incubation. After 60-second erosion, the samples were removed, rinsed with distilled water and placed in a new Eppendorf tube.

**Profilmetric Analysis (Experiment 1)**

Enamel loss was quantitatively determined by profilometry (Perthometer S2, Mahr, Göttingen, Germany) after application of
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Fig. 1. Cumulative enamel loss (μm) and regression lines in the different groups after application of fluoride solutions and after 1-, 5- and 10-min erosion. × = Control (R = 0.95, p < 0.001); ■ = TiF₄, pH 1.2 (R = 0.08, p = 0.08); □ = TiF₄, pH 3.5 (R = 0.94, p < 0.001); ○ = NaF, pH 1.2 (R = 0.87, p < 0.001); △ = NaF, pH 3.5 (R = 0.87, p < 0.001). At t = 10-min erosion, TiF₄ at pH 1.2 was significantly different (marked with *) from all other groups, which in turn were not significantly different from each other.

the fluoride solutions and after 1, 5 and 10 min of erosion. Prior to the experiment, three baseline surface profiles were obtained as references for the calculation of enamel loss. After application and after 1-, 5- and 10-min erosion, the tape was removed and 3 profiles were recorded at exactly the same sites as for the baseline measurement, using identification marks made on the reference surface with a scalpel, which allowed accurate repositioning of the stylus. The spacing and length of the profiles were 250 and 1,500 μm, respectively. The average depth of enamel loss relative to the baseline surface profiles was calculated by Perthometer Concept 7.0 software (Mahr, Göttingen, Germany).

Determination of Calcium Loss (Experiment 2)
Calcium dissolved from the enamel samples during erosion was analysed by continuum source atomic absorption spectroscopy (ContraA 300, Analytik Jena AG, Jena, Germany, air/acetylene flame) at 422.7 nm. The amount of calcium released into the acid was determined in each 1-min acid fraction [Wiegand et al., 2008]. As phosphate might depress the sensitivity for calcium, 0.25% strontium chloride was added to the sample solutions.

X-Ray EDS and SEM (Experiment 3)
The Ti and F surface composition of the fluoride-treated but not eroded samples was obtained by EDS and SEM (SUPRA 50 VP and Genesis, Carl Zeiss NTS GmbH, Oberkochen, Germany). After pretreatment, the samples were desiccated for 4 weeks in blue silica gel [Schmidlin et al., 2001, 2002] in a vacuum evaporator directly after treatment with the respective fluoride solution. EDS measurement was performed in 6 specimens per group. 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 were analysed stoichiometrically.

For SEM examination of enamel surfaces pretreated with fluoride or water, 2 samples from each group were desiccated as described above, sputter-coated with gold for 60 s and then examined at 10–20 kV.

For SEM examination after erosion, 2 samples per group were desiccated as described above and infiltrated with an isobornyl methacrylate resin (Technovit 720 VLC, Heraeus Kulzer, Switzerland) as described elsewhere [Lottanti et al., 2009]. After that, cross sections of the enamel samples were prepared, sputter-coated with gold for 60 s and examined in the scanning electron microscope in backscattered electron mode at 20 kV.

Statistical Analysis
Cumulative enamel loss (experiment 1) and cumulative calcium release (experiment 2) were analysed by repeated measures analysis of variance (ANOVA) followed by Tukey tests. Moreover, cumulative enamel loss and calcium release at t = 10 min were analysed by one-way ANOVA followed by Fisher’s PLSD and Dunnnett’s post-hoc tests.

Finally, linear regressions were performed for both cumulative enamel loss and calcium release (Statistica 5.1, Statsoft Inc., Tulsa, Okla., USA). The data of EDS analysis (experiment 3) were analysed separately for each element by one-way ANOVA and Dunn’s multiple comparison tests (Graph Pad InStat version 3.0 for Windows, Graph Pad Software, San Diego, Calif., USA). The level of significance was set at p < 0.05.

Results
Experiment 1
Figure 1 illustrates the cumulative enamel loss (μm, experiment 1) in the different groups after application of the fluoride solutions and after 1-, 5- and 10-min erosive treatment. Except for TiF₄ at pH 1.2, a significant linear correlation between enamel loss and erosion time could be observed in all groups. Repeated-measures ANOVA revealed significant differences among the groups and among the respective time points (application, 1-, 5- and 10-min erosion, R = 0.86, p < 0.001). The application of TiF₄ at pH 1.2 and pH 3.5 resulted in no enamel wear (–0.07 ± 0.22 and –0.002 ± 0.04 μm, respectively; mean ± SD), while NaF pretreatment led to 0.51 ± 0.31 μm (pH 1.2) and 0.16 ± 0.39 μm (pH 3.5) enamel loss. Repeated-measures ANOVA and one-way ANOVA at t = 10 min showed that only TiF₄ at pH 1.2 reduced erosive enamel loss significantly compared to the control and all other groups, which in turn were not significantly different from the control or from each other.

Experiment 2
Cumulative calcium loss after 1–10 min of erosion (experiment 2) is presented in figure 2. In all groups there was a significant linear correlation between calcium release and erosion time. Repeated-measures ANOVA showed significant differences among the groups and
among the time points ($R = 0.96$, $p < 0.001$). Tukey’s tests revealed that all fluoride groups except NaF at pH 3.5 were significantly different from the control over time. Thereby, TiF$_4$ and NaF at pH 1.2 were equally effective in reducing calcium release ($p > 0.05$). At $t = 10$ min, calcium release was significantly reduced by TiF$_4$ and NaF at pH 1.2, but not by TiF$_4$ and NaF at pH 3.5.

**Experiment 3**

The results of EDS analysis are presented in table 1. Treatment with TiF$_4$ at pH 1.2 led to a significant increase in Ti compared to all other groups. Elemental surface composition in samples treated with TiF$_4$ at pH 3.5 was not significantly different from controls. The application of NaF led to a significant increase in F, which was distinctly higher for the solution at pH 1.2 than for the solution at pH 3.5.

SEM images of the enamel surfaces after pretreatment with the fluoride solutions or water, respectively, are shown in figure 3. While pretreatment with TiF$_4$ at pH 1.2 (fig. 3a) led to the formation of a surface coating, the surface pretreated with NaF at pH 1.2 (fig. 3b) appeared to be distinctly demineralised. Samples pretreated with TiF$_4$ and NaF at pH 3.5 (fig. 3c, d) presented a slightly demineralised surface compared to the control sample (fig. 3e).

Figure 4 presents the cross-sectional SEM images of enamel samples after 10 min of erosion. Samples pretreated with TiF$_4$ at pH 1.2 (fig. 4a) exhibit a subsurface demineralisation below the surface coating, while all other groups (fig. 4b–e) show distinctly demineralised surfaces.

**Discussion**

The data of the present study show that TiF$_4$ at pH 1.2 but not at pH 3.5 induces the formation of a glaze-like layer protecting from surface loss, indicating that the protective capacity of TiF$_4$ is related to the low pH of the native solution.

As in previous experiments [Magalhães et al., 2008a, b], the fluoride solutions were applied only once for 60 s to simulate a realistic application time under clinical conditions. Bovine enamel was used as a substrate as it is widely used in erosion research and has chemical and mechanical properties similar to human enamel. However, for extrapolating in vitro data to the clinical situation it should be taken into account that the susceptibility to erosion might differ between bovine and human enamel [Rios et al., 2006; Attin et al., 2007]. Moreover, TiF$_4$ pretreatment might be more effective in bovine than in human enamel. Hove et al. [2007b] showed that the protection of TiF$_4$ pretreatment against erosive calcium loss was significantly better in bovine compared to human enamel. In the present study, different methods for analysing enamel erosion were applied to investigate both the mechanical (profilometry, SEM images) and chemical (calcium analysis and EDS) effect of the fluoride solutions on enamel and enamel erosion. To allow for a precise profilometric measurement [Attin, 2006], polished instead of natural enamel surfaces were used, although

![Fig. 2.](image-url)

**Table 1.** Titanium and fluoride surface composition in the different groups

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>Ti</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>$0.1 \pm 0.1^a$</td>
<td>$0.0 \pm 0.1^a$</td>
</tr>
<tr>
<td>TiF$_4$, pH 1.2</td>
<td>$0.8 \pm 0.4^{b, c}$</td>
<td>$2.5 \pm 1.2^b$</td>
</tr>
<tr>
<td>TiF$_4$, pH 3.5</td>
<td>$0.4 \pm 0.2^{a, b}$</td>
<td>$0.3 \pm 0.1^{a, b}$</td>
</tr>
<tr>
<td>NaF, pH 1.2</td>
<td>$13.3 \pm 1.0^{a}$</td>
<td>$0.0 \pm 0.0^a$</td>
</tr>
<tr>
<td>NaF, pH 3.5</td>
<td>$1.5 \pm 0.9^{b, c}$</td>
<td>$0.1 \pm 0.1^a$</td>
</tr>
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Statistical analysis was done by non-parametric ANOVA followed by Dunn’s multiple comparisons test as the standard deviations in the groups were significantly different. Within each element, significant differences were marked with distinct letters.
Fig. 3. SEM images of enamel surfaces after pretreatment with the different fluoride solutions or water, respectively, ×80,000 magnification. a TiF₄ at pH 1.2. b NaF at pH 1.2. c TiF₄ at pH 3.5. d NaF at pH 3.5. e Control.
Fig. 4. Cross-sectional SEM images of enamel samples after 10-min erosion. Enamel surfaces are covered and partly penetrated by the acrylic resin. ×10,000 magnification, bar = 2 μm. a TiF₄ at pH 1.2. b NaF at pH 1.2. c TiF₄ at pH 3.5. d NaF at pH 3.5. e Control.
polished surfaces might show a slightly higher susceptibility to erosion than natural surfaces [Ganss et al., 2000]. For experiment 1 and experiments 2/3 the volume of the fluoride solutions was adjusted to the surface of the specimens to allow for comparison of the results of the different analyses. Moreover, the specimens were incubated with an excess of acid, which was renewed after each 60 s, providing constant pH levels.

As seen from the results of profilometric analysis and SEM images, the application of TiF₄ at pH 1.2 did not lead to enamel surface loss, despite the very low pH, but to an almost dense surface layer. In contrast, SEM images of a previous study by Magalhães et al. [2008a] did not show the formation of a glaze-like surface layer. In this study, the 4% TiF₄ solution was applied with a microbrush, while the solutions in the present study were pipetted onto the enamel surface. It might be speculated that the application by microbrush leads to a wear of the surface rather than allowing for the formation of the glaze-like surface layer.

In accordance with previous studies [Clarkson and Wefel, 1979; Chevitarese et al., 2004; Magalhães et al., 2008a], EDS analysis showed that this layer is very rich in titanium. Titanium ions might play an important role for the protective capability of TiF₄ as titanium can bind to enamel surfaces and penetrate into sound or demineralised enamel [Clarkson and Wefel, 1979; Chevitarese et al., 2004]. It is assumed that the coating found after the application of TiF₄ is composed of organometallic complexes. This hypothesis is confirmed by Mundorff et al. [1972], who showed that the formation of the glaze-like layer was distinctly decreased on organic reduced enamel. Alternatively it is speculated that titanium phosphate compounds are formed [Ribeiro et al., 2006] or that titanium can substitute for calcium in the apatite lattice [Ribeiro et al., 2008a]. This hypothesis is confirmed by Mundorff et al. [1972], who showed that the formation of the glaze-like layer was distinctly decreased on organic reduced enamel. Alternatively it is speculated that titanium phosphate compounds are formed [Ribeiro et al., 2006] or that titanium can substitute for calcium in the apatite lattice [Leadley et al., 1997], leading to higher acid resistance.

In contrast to titanium, the surface fluoride concentration was only slightly increased compared to the control. However, in a previous study it was shown that even after the application of 4% TiF₄ solution the fluoride concentration at the surface amounted to only 2% [Magalhães et al., 2008a].

While enamel surface loss was reduced almost completely by the application of TiF₄ at pH 1.2, the reduction of calcium loss was only 30% after 1 min and 16% after 10 min of erosion. From the cross-sectional SEM images after erosion it becomes evident that TiF₄ provides only superficial mechanical protection and cannot prevent the formation of subsurface demineralisation below the glaze-like surface layer completely. The thickness of this subsurface demineralisation (~5 µm) is in the range of the erosive surface loss (measured profilometrically) of all other groups. Previous studies showed by SEM that the layer on enamel surfaces after treatment with TiF₄ is not homogeneous [Wei et al., 1976; Büyükyilmaz et al., 1995, 1997]. The results of the present study suggest that this layer presents some inhomogeneities or microcracks which allow for the penetration of acid into the subsurface enamel layer and, thus, for subsurface demineralisation and the release of calcium. Further studies will need to analyse whether reapplication of TiF₄ might allow for a penetration of the solution into the subsurface zone, thus decreasing further dissolution. Moreover, the abrasion resistance of the superficial layer has to be evaluated, as dental hard tissues are exposed not only to erosive but also to abrasive influences, such as toothbrushing, under clinical conditions.

In contrast to the TiF₄ solution at pH 1.2, the TiF₄ solution at pH 3.5 failed to reduce enamel surface loss and calcium release. In accordance with the absent surface layer after the application of TiF₄ at pH 3.5, the titanium surface concentration was only slightly increased compared to the control. Similar to the samples pretreated with water (control) or NaF, the cross-sectional SEM images of eroded specimens pretreated with TiF₄ at pH 3.5 revealed a frayed and demineralised surface after erosion. These results indicate that the protective capability of TiF₄, in particular the formation of the glaze-like surface coating, might be highly dependent on the pH of the solution.

In the present study, NaF solution at pH 1.2 was able to reduce calcium release by 38% after 1-min erosion and by 17% after 10-min erosion, but not enamel surface loss significantly, as seen from profilometry and the cross-sectional SEM images. The protective effect of NaF is related to the formation of CaF₂-like surface precipitates, which were shown to be significantly enhanced under acidic conditions as well as with increasing length of fluoride exposure and fluoride concentration [Øgaard, 2001; Ganss et al., 2007]. This loosely bound fluoride might protect the surface to a certain extent against demineralisation as it acts as a reservoir for fluoride which facilitates the reprecipitation of minerals by forming fluorapatite or fluoroxyapatite, thereby preventing further loss of mineral ions [Rølla et al., 1993].

However, even though EDS analysis revealed higher amounts of fluoride in the samples treated with NaF, especially at pH 1.2, SEM pictures did not show the deposition of loosely bound fluoride in the form of globular precipitates. It might be speculated whether the loosely
bound fluoride formed at pH 1.2 is different from fluoride typically found in globular precipitates or if fluoride is structurally bound in the outermost enamel rather than being present in CaF₂ precipitates.

In contrast to the present study, Schlueter et al. [2007] found a 2.2% NaF solution at pH 1.2 to reduce enamel erosion to approximately 50% in a 5-day de- and remineralisation cycle. The different results might be explained by the frequency of fluoride treatment. While in the present study the fluoride solutions were applied only once for 60 s, Schlueter et al. [2007] applied the NaF solution daily for 5 min. The frequent application of NaF might lead to better protection against enamel erosion.

From the results of the present study it can be concluded that the efficacy of TiF₄ to prevent erosive surface loss is related to the glaze-like surface layer, which is formed when TiF₄ is applied at the native pH (1.2) of the solution. Thus, the working hypotheses that TiF₄ at pH 1.2 and 3.5 is equally effective in reducing erosion and that TiF₄ and NaF are equally effective, independently of the pH, are rejected.

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