Protection of short-time enamel erosion by different tetrafluoride compounds

Wiegand, A; Laabs, K A; Gressmann, G; Roos, M; Magalhães, A C; Attin, T
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Abstract

OBJECTIVE: This in vitro study aimed to analyse the protective effect of differently concentrated titanium (TiF(4)), zirconium (ZrF(4)) and hafnium (HfF(4)) tetrafluoride on enamel erosion. METHODS: Polished enamel surfaces of 36 bovine crowns were covered with tape leaving 4 enamel windows each 3mm in diameter exposed. The crowns were randomly assigned to six groups (each n=6) and pretreated with 4% TiF(4), 10% TiF(4), 4% ZrF(4), 10% ZrF(4), 4% HfF(4) or 10% HfF(4) for 4 min (first window), 10 min (second window) or 15 min (third window). The fourth window of each crown was not pretreated and served as control. Erosion was performed stepwise with 1% HCl (pH 2) in five consecutive intervals of each 15 s (total 75 s). Enamel dissolution was quantified by colorimetric determination of phosphate release into the acid. For each tooth, cumulative phosphate loss of enamel pretreated with one of the tetrafluoride compounds was calculated as percentage of the respective control and statistically analysed using two-way ANOVA. RESULTS: Enamel erosion was significantly reduced by TiF(4), ZrF(4) and HfF(4) application. Cumulative phosphate loss (mean % of control, 75s erosion) after 4-15 min application was significantly lower for 4% ZrF(4) (7-11%), 10% ZrF(4) (2-6%), 4% HfF(4) (11-9%) and 10% HfF(4) (12-16%) compared to 4% TiF(4) (42-27%) and 10% TiF(4) (54-33%). Only for 4% and 10% TiF(4), phosphate loss decreased with increasing duration of application, but also increased with increasing acid intervals. CONCLUSION: TiF(4), ZrF(4) and HfF(4) might protect enamel against short-time erosion, but protection was more enhanced by ZrF(4) and HfF(4) compared to TiF(4) application overtime.
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Methods: Polished enamel surfaces of 36 bovine crowns were covered with tape leaving 4 enamel windows each 3 mm in diameter exposed. The crowns were randomly assigned to 6 groups (each n = 6) and pretreated with 4% TiF₄, 10% TiF₄, 4% ZrF₄, 10% ZrF₄, 4% HfF₄ or 10% HfF₄ for 4 min (1st window), 10 min (2nd window) or 15 min (3rd window). The 4th window of each crown was not pretreated and served as control. Erosion was performed stepwise with 1% HCl (pH 2) in five consecutive intervals of each 15 s (total 75 s). Enamel dissolution was quantified by colorimetric determination of phosphate release into the acid. For each tooth, cumulative phosphate loss of enamel pretreated with one of the tetrafluoride compounds was calculated as percentage of the respective control and statistically analysed using two-way ANOVA.

Results: Enamel erosion was significantly reduced by TiF₄, ZrF₄ and HfF₄ application. Cumulative phosphate loss (mean % of control, 75 s erosion) after 4-15 min application was significantly lower for 4% ZrF₄ (7-11%), 10% ZrF₄ (2-6%), 4% HfF₄ (11-9%) and 10% HfF₄ (12-16%) compared to 4% TiF₄ (42-27%) and 10% TiF₄ (54-33%). Only for 4% and 10% TiF₄, phosphate loss decreased with increasing duration of application, but also increased with increasing acid intervals.

Conclusion: TiF₄, ZrF₄ and HfF₄ might protect enamel against short-time erosion, but protection was more enhanced by ZrF₄ and HfF₄ compared to TiF₄ application overtime.

Key words: enamel, erosion, fluoride
**Introduction**

Topical fluoridation in form of acidic sodium, amine or stannous fluoride is able to decrease enamel or dentine erosion, but requires intensive and frequent application to reduce erosive demineralization substantially.\(^1\)-\(^3\) Recently, research into dental erosion focused on the anti-erosive potential of titanium tetrafluoride (TiF\(_4\)), which is suggested to be more effective in inhibition of enamel erosion or carious demineralisation than sodium, stannous or amine fluoride.\(^4\)-\(^7\)

Hove et al.\(^5\) reported that TiF\(_4\) (0.5 M F) reduced enamel erosion almost completely (88%), while SnF\(_2\) and NaF treatment resulted in only 50% or 25% protection, respectively. Schlüter et al.\(^4\) investigated the influence of 1.64 % TiF\(_4\) and 2.2% NaF (both pH: 1.2) in a cyclic de- and remineralization protocol over 5 days. TiF\(_4\)-treated enamel samples showed significantly less mineral loss than samples of the NaF group. TiF\(_4\) was also more effective than amine or sodium fluoride in preventing enamel calcium loss by an artificial caries solution.\(^7\)

The protective effect of TiF\(_4\) is attributed to the mechanical protection of the surface by formation of a glaze-like layer as well as to an increased fluoride uptake following TiF\(_4\) application. Thus, TiF\(_4\) might be more protective against the acid action than the calcium fluoride precipitate formed by sodium or amine fluoride treatment.

Early studies in caries prevention suggested that not only TiF\(_4\) but also other tetrafluoride compounds might reduce the formation of artificially created caries lesions. Shrestha et al.\(^8\) demonstrated that TiF\(_4\) and also zirconium (ZrF\(_4\)) and hafnium tetrafluoride (HfF\(_4\)) reduced enamel dissolution in an acetic acid buffer (pH 4) significantly. Similarly, Mühlemann et al.\(^9\) found that enamel pretreatment with ZrF\(_4\) lead to 53% reduction of calcium and phosphate loss induced by an artificial caries solution.
These promising results suggest that tetrafluorides other than TiF$_4$ might also exhibit protective potential against erosive demineralisation. Hence, the aim of the present pilot study was to evaluate the effect of TiF$_4$, ZrF$_4$ and HfF$_4$ on short-term enamel erosion.

**Methods**

**Sample preparation**

Thirty-six intact crowns were obtained from freshly extracted bovine incisors and embedded in acrylic resin (Paladur, Heraeus Kulzer, Hanau, Germany). The labial surfaces were ground flat and polished with water-cooled carborundum discs (500-1200 grit, Water Proof Silicon carbide Paper, Stuers, Erkrat, Germany). The thickness of the removed outermost enamel layer amounted to 230-250 µm and was controlled with a micrometer (Digimatic®, Micrometer, Mitutoyo, Tokyo, Japan). The size of the polished enamel area amounted to approximately 9 x 9 mm. A masking adhesive rubber tape (Coroplast, Wuppertal, Germany) was applied firmly on the polished surface, and four circular holes were punched into the tape leaving four enamel windows each 3 mm in diameter exposed. This tape was fixed to the crown surface throughout the whole experiment. The crowns were randomly assigned to 6 groups with 6 crowns each and stored in distilled water until required.

**Fluoride compounds**

In the experiment, the following fluoride compounds were used: 4% TiF$_4$ (pH <1), 10% TiF$_4$ (pH <1), 4% ZrF$_4$ (pH 1.6), 10% ZrF$_4$ (pH 1.4), 4% HfF$_4$ (pH 1.6) or 10% HfF$_4$ (pH 1.2). The respective TiF$_4$, ZrF$_4$ and HfF$_4$ solutions were prepared by mixing titanium tetrafluoride, zirconium tetrafluoride or hafnium tetrafluoride (impurity: 1% zirconium) powder (Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany) with distilled water. The solutions were
remixed for 120 s prior to application on an orbital shaker (Vortex, Schütt Labortechnik, Germany).

Erosion experiment

Prior to acid exposure, the crowns were pretreated with the respective fluoride agent for 4 min (1st window), 10 min (2nd window) or 15 min (3rd window). Each 8 µl of the fluoride solution was applied to respective window for 4, 10 or 15 min, while the 4th window of each crown was not pretreated (0 min) and served as negative control. After application of the fluoride agents for 4, 10 or 15 min, the crowns were rinsed with distilled water and carefully dried by air pressure.

Erosion was performed with 1% hydrochloric acid (pH 2) in five consecutive intervals of each 15 s. Therefore, 10 µl of the acid was pipetted to each enamel window and applied under constant agitation. During the erosion of each window, the remaining windows were covered by tape. After each 15 s acid exposure, 5 µl of the acid was submitted to the phosphate analysis. Prior to the next acid exposure, the crowns were rinsed with distilled water for 10 s and carefully dried by air pressure.

Phosphate analysis

Enamel dissolution in the different experimental groups was assessed by colorimetric analysis of phosphate release into the acid. Phosphate loss was determined in each 15 s acid fraction using a malachite green procedure described in detail previously. In summary, malachite green reacts with phosphate to a coloured complex which can be determined at λ = 650. For this purpose, 0.045 g malachite green dissolved in 100 ml aqua bidest. was admixed to 12.69 g ammonium molybdate, which was dissolved in 300 ml HCl (4 mol/l). The reagent was stirred for 30 min afterwards and filtered. For colorimetric phosphate determination, 5 µl of each 15 s fraction (10 µl acid) was admixed to 200 µl of the reagent.
Individual standard curves were obtained by admixing diluted samples of the standardized phosphate solution to the acid. The lowest standard of the calibration curve (0.117 nmol phosphate/10 µl acid) was considered as threshold for precision of the measurement.

Statistical analysis
Phosphate loss of windows 1 to 4 of each tooth was measured as described above. Values below the threshold of precise phosphate analysis were considered as not detectable and set as zero.

For each tooth, cumulative phosphate loss of each window after 5 x 15 s (75 s) acid exposure was calculated. To consider individual variation of enamel solubility of different teeth, cumulative phosphate release of windows 1 to 3 was calculated as percentage of the respective negative control (4th window, without pretreatment). Cumulative phosphate loss (mean % of control ± S.D.) was statistically analysed by two-way ANOVA considering both the tetrafluoride agent and the duration of tetrafluoride application (4 min, 10 min and 15 min) as independent variables. Within each tetrafluoride group, one-way ANOVA was performed for each application time separately. ANOVA was followed by Scheffé post-hoc tests.

Additionally, phosphate release of windows 1 to 4 into each 15 s acid fraction was analysed by repeated measurement ANOVA to check whether phosphate loss changed with increasing acid intervals. The overall level of significance was set at p < 0.05.

Results
Phosphate release into the acid was significantly reduced by enamel pretreatment with TiF₄, HfF₄ and ZrF₄. Mean cumulative phosphate release (% of control ± standard deviation) after application of the different tetrafluoride agents is shown in Figure 1.
ANOVA revealed the cumulative phosphate loss to be affected by the kind of tetrafluoride pretreatment (p < 0.001). Moreover, tetrafluoride pretreatment and duration of application showed significant interactions (p = 0.0323). Only 4% TiF₄ and 10% TiF₄ showed a significant decrease of cumulative phosphate loss with increasing duration of application (p = 0.0436 and p = 0.0067, respectively). However, HfF₄ and ZrF₄ showed no significant differences between cumulative phosphate loss after 4, 10 and 15 min application time (p > 0.09).

Generally, the reduction of phosphate release by tetrafluoride application was significantly higher for HfF₄ and ZrF₄ than for TiF₄ (Figure 1). Efficacy of the agents was not affected (p > 0.3) by their concentration (4% or 10%). Cumulative phosphate release of enamel pretreated with HfF₄ and ZrF₄ was not significantly different (p > 0.06). This effect was also observed when data were split for the different application times. Between-group comparisons of cumulative phosphate loss after 4 min, 10 min and 15 min application time are presented in Table 1. TiF₄ was significantly less effective in reducing phosphate loss and, thus, enamel erosion compared to HfF₄ and ZrF₄ when applied for 4 or 10 min. However, these differences were not evident after 15 min, when only 10% TiF₄ - 10% ZrF₄ were significantly different.

Mean phosphate release (nmol/mm²) in each 15 s acid fraction is shown in Table 2. Repeated measurement ANOVA showed that the reduction of phosphate loss by 4% and 10% TiF₄ decreased with increasing acid intervals (p < 0.001). Thereby, phosphate loss after 75 s acid contact (5th acid interval) was two- to threefold higher compared to phosphate loss after the first acid interval (15 s acid contact). In contrast, in groups “ZrF₄” and “HfF₄” as well as in the control windows, phosphate release into each 15 s acid fraction remained constant over time and did not show significant differences.
Discussion

This pilot study has shown that topical application of TiF$_4$, ZrF$_4$ and HfF$_4$ is effective in protecting enamel from phosphate loss during short-term erosion by hydrochloric acid.

Bovine crowns were used due to their larger surface area compared to human teeth, which allows for analysis of the test (windows 1-3) and control (window 4) groups on the same teeth. Bovine enamel is widely used in erosion research as its chemical and mechanical properties are similar to human enamel, although bovine samples might be more susceptible to erosion than human enamel specimens. However, a recent study by Hove et al. found a significant difference in the protective effect of TiF$_4$ on human and bovine enamel. Thereby, the application of TiF$_4$ lead to significantly less erosion in bovine compared to human enamel, while untreated bovine and human samples offered the same degree of erosion. These results should be taken into account for extrapolation from in vitro data to the clinical situation.

Short-term erosion of the samples was performed by strong hydrochloric acid to simulate the reflux or vomiting attacks with the exposure of gastric juice in the oral cavity. The estimated etching depth after only five successive 15 s acid contacts might be too small to be analysed by profilometry or microradiography. Thus, mineral loss was analysed chemically by a colorimetric method, which allows for determination of minimal amounts of phosphate in acids. The precision of the method was described previously. Several previous studies used calcium and phosphate analysis as indirect technique for dissolution analysis of TiF$_4$-treated samples.

The protective effect of TiF$_4$ is attributed to the acid-resistant surface coating formed by the fluoride-metal complex, which might lead to a mechanical protection of the surface. Furthermore, the application of TiF$_4$ might lead to an increase in the enamel fluoride content. The TiF$_4$ solution in itself is very acidic and might induce a demineralization of the enamel surface and the formation of HF. This might enhance the depth of penetration of fluoride ions and promote the formation of CaF$_2$. The titanium ions might play an important role as they...
might substitute calcium in the apatite lattice and show a strong tendency to complex with phosphate groups, forming stable titanium dioxide layer\textsuperscript{14-16}. Moreover, it is suggested that titanium interacts with organic components of the enamel surface, leading to an increased fluoride uptake by enamel.\textsuperscript{14,17}

However, Mundorff et al.\textsuperscript{14} suggested that hafnium and zirconium might be unable to form an acid-stable surface glaze. In contrast, by SEM analysis it could be shown that pretreatment of enamel surface with different polyvalent ions followed by application of acidulated phosphate fluoride lead to the formation of an amorphous coating on the enamel surface. Thereby, zirconium chloride and ferric chloride lead to the formation of a relatively thick surface coating, while titanium chloride pretreatment showed a thinner and fragile surface coating.\textsuperscript{18}

Thus, it might be assumed, that HfF\textsubscript{4} and ZrF\textsubscript{4} application might also lead to a mechanical protection of the enamel surface,\textsuperscript{18,19} which shows a similar mode of action as the TiF\textsubscript{4} glaze-like layer. Moreover, it is suggested that the hafnium and zirconium ions might also act chemically, as it is known that metal ions lead to an increased fluoride retention into enamel due to their ability to complex with fluoride.\textsuperscript{20} Although HfF\textsubscript{4}- and ZrF\textsubscript{4}-treated samples are not significantly different in the present study, it seems unlikely that the protective efficacy of HfF\textsubscript{4} is affected by zirconium impurities, as the fraction of zirconium in the hafnium powder amounts to 1% only.

In general, the protection of enamel erosion by TiF\textsubscript{4}, HfF\textsubscript{4} and ZrF\textsubscript{4} is in the range of the protective effect reported for TiF\textsubscript{4} in previous studies. In these studies, the reduction of enamel erosion of short-term demineralised samples (2-4 min, hydrochloric acid, pH 2) amounted to 76-88%.\textsuperscript{5,12} However, as the present pilot study focused only on the effects of the tetrafluorides to short-term erosion, it has to be analysed whether the increased protective capacity of HfF\textsubscript{4} and ZrF\textsubscript{4} compared to the TiF\textsubscript{4} could also be evaluated in long-term eroded samples. Shrestha et al.\textsuperscript{8} analysed the long-term efficacy of 1% TiF\textsubscript{4}, 2% HfF\textsubscript{4} and 2% ZrF\textsubscript{4} solutions on enamel submitted to 15 min demineralisation by an acetic acid buffer (pH: 4). In
contrast to the results of the present study, TiF₄ pretreatment lead to a significant better reduction of enamel solubility (95%) compared to HfF₄ (82%) and ZrF₄ (85%). The different results might be related to the higher pH of the acidic buffer and the lower concentration of the tetrafluoroide solutions in the study by Shrestha et al.⁸. From these results it might be speculated that, at concentrations lower than 4%, TiF₄ might be more effective than HfF₄ and ZrF₄, especially at increasing pH-values of the demineralising solution.

In agreement to previous studies, the tetrafluoride compounds were applied at a concentration of 4%.²¹-²³ It was speculated, that the application of 10% HfF₄-, ZrF₄- and TiF₄-solution might increase their protective capability. Although the results of Bükükylmaz et al.²³ indicate that the thickness and texture of the glaze-like layer is dependent on the concentration of the tetrafluoride solution, this pilot study did not show differences in the protective capability of 4% and 10% tetrafluoride solutions. Only for the TiF₄ samples, the reduction of phosphate loss was dependent on the duration of application. Although there is a lack of studies evaluating the influence of the duration of application on the erosion-inhibiting effect, the results of Exterkate and ten Cate²⁴ indicate that the efficacy of titanium fluorides is enhanced by frequent application. Further studies have to evaluate if the efficacy of tetrafluorides can be enhanced by frequent, but short-time application of the solutions. This might be an interesting approach to increase the protective impact of tetrafluorides, especially when considering that the efficacy of TiF₄ was gradually reduced during acid treatment in the present study, while the erosion protective effects of HfF₄ and ZrF₄ remained stable.

However, from the results of this pilot study it is concluded that not only TiF₄, but also ZrF₄ and HfF₄ application provides a potential treatment option for short-time enamel erosion. Thereby, enamel pretreatment with 4% solutions applied for 4 min offered promising results, which were not significantly enhanced by increasing the concentration of the agent or the duration of application. Further research is necessary to evaluate in the effect of tetrafluorides, in particular of ZrF₄ and HfF₄, on pellicle-covered enamel, but also on dentine surfaces.
Thereby, the efficacy of tetrafluoride solutions with lower concentration as well as the long-time effects of the tetrafluoride protection have to be evaluated. Finally, more information about the protective effects of tetrafluoride treatment in relation to abrasive influences is necessary.
References


Figure 1:
Cumulative phosphate loss (mean % of control ± S.D.) after 75 s of erosion, when the TiF$_4$, ZrF$_4$ and HfF$_4$ solutions were applied on enamel windows for 4 min, 10 min or 15 min. Tetrafluoride groups marked with the same capital letter are not significantly different. Within each tetrafluoride group, significant differences among cumulative phosphate release after the different application times (4, 10 and 15 min) are marked by different small letters.
Table 1:
Between-group comparisons of cumulative phosphate loss (p-values) after 4 min, 10 min and 15 min application time and over the complete experiment (overall, 75 s of erosion). Scheffé post-hoc p-values marked by * were statistically significant different.
<table>
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<th>Tetrafluoride agent</th>
<th>Application time (min)</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; acid fraction</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; acid fraction</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt; acid fraction</th>
<th>4&lt;sup&gt;th&lt;/sup&gt; acid fraction</th>
<th>5&lt;sup&gt;th&lt;/sup&gt; acid fraction</th>
<th>Cumulative (after 75 s)</th>
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<tr>
<td>4% TiF₄</td>
<td>control</td>
<td>1.63 ± 0.43</td>
<td>1.57 ± 0.48</td>
<td>1.60 ± 0.43</td>
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<td>2.23 ± 0.51</td>
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<td>0.39 ± 0.36</td>
<td>1.47 ± 1.47</td>
</tr>
</tbody>
</table>

Table 2:

Mean phosphate loss (nmol/mm²) ± S.D. in each 15 s acid fraction (10 µl acid) and cumulative phosphate loss (nmol/mm²) ± S.D. after 75 s acid exposures in the different groups.