Effect of TiF4, ZrF4, HfF4 and AmF on erosion and erosion/abrasion of enamel and dentin in situ

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Abstract

OBJECTIVE: This in situ study aimed to analyse the impact of different tetrafluorides (TiF(4), ZrF(4) and HfF(4)) and AmF on erosion and erosion plus abrasion of enamel and dentin. DESIGN: Ten volunteers took part in this crossover and double-blind study performed in 8 phases of each 3 days. In each phase, 2 bovine enamel and 2 dentin specimens were fixed in intraoral appliances. One enamel and one dentin sample were pretreated once with TiF(4), ZrF(4), HfF(4) or AmF (all 0.5M F) for 60s, while the other samples remained unfluoridated and served as control. Then, all samples were subjected to either erosion only (4 times/day, 90 s) or to erosion and abrasion (2 times/day, 30 s/sample). Toothbrushing abrasion was performed 90 min after the first and last erosion with an electrical toothbrush and fluoridated toothpaste at 1.2N. After 3 days, enamel and dentin loss was assessed by profilometry (microm) and analysed by repeated measures ANOVA and paired t-test (p<0.05).

RESULTS: All fluoride solutions reduced enamel and dentin loss significantly compared to the controls. Generally, eroded samples showed less wear than eroded and abraded samples. The protective potential of the fluorides was not significantly different and was only slightly, but mostly not significantly, decreased by abrasion. The protective effect of the fluoride solutions was similar in enamel and dentin.

CONCLUSION: Tetrafluorides and AmF are able to reduce erosion and erosion plus abrasion in situ and are almost equally effective.
Effect of TiF$_4$, ZrF$_4$, HfF$_4$ and AmF on erosion and erosion/abrasion of enamel and dentin in situ

Running title: Tetrafluorides and erosion

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Conclusion: Tetrafluorides and AmF are able to reduce erosion and erosion plus abrasion in situ and are almost equally effective.

Key words
Enamel, dentin, erosion, fluoride
Introduction

In recent years, the erosion-inhibiting effect of metal-containing fluoride compounds, such as titanium tetrafluoride (TiF₄), has gained increasing attention as they were mostly found to be more effective in reducing enamel erosion than sodium or amine fluoride.¹⁻³ However, considering that most of the studies were performed in vitro and concentrated mainly on the effects on enamel but not on dentin erosion³⁻⁶, the protective effect of tetrafluoride compounds on dental erosion is still questionable and requires confirming data from in situ studies and clinical trials.

As yet, only three studies evaluated the effect of TiF₄ on enamel erosion in situ, but showed conflicting results. Magalhães et al.⁷,⁸ showed that the application of a 4% TiF₄ solution reduced the erosive softening of enamel, probably by forming a titanium dioxide glaze-like layer. On the other hand, the single application of TiF₄ was not able to reduce erosive enamel wear during a 5-day in situ period.⁹ In contrast, Hove et al.⁹ showed that TiF₄ reduced the enamel etching depth nearly completely during a 9 day study period, when the 1.5% TiF₄ solution was applied each third day. Due to the fact that the tested tetrafluoride solutions usually have high fluoride concentrations, it would be more realistic to apply them only once, simulating the professional application.

However, under clinical conditions, dental hard tissues are exposed not only to erosive but also to abrasive influences, such as toothbrushing. Ideally, the precipitates found after fluoridation should resist abrasive forces to exhibit a long-lasting protective effect against erosive acids. However, it was shown that brushing abrasion removed the calcium-fluoride like precipitates formed on enamel after application of NaF and AmF partially,¹⁰ which might decrease the anti-erosive efficacy. Moreover, it was shown in vitro that brushing abrasion led to slightly, although not significantly higher wear in TiF₄-treated enamel compared to untreated controls.¹¹ It was speculated that the superficial glaze-like layer might be less resistant to abrasive forces and the subsurface zone might be more prone to wear.¹²

Thus, the present study aimed to analyse the efficacy of a TiF₄ on enamel and dentin erosion and combined erosion–abrasion. As it was recently shown that zirconium tetrafluoride (ZrF₄) and hafnium tetrafluoride (HfF₄) were also effective to reduce enamel and dentin erosion,⁵,¹³
equimolar solutions of TiF$_4$, ZrF$_4$ and HfF$_4$ were used in this study and compared with an amine fluoride (AmF) solution equimolar with respect to fluoride.

The null hypotheses tested were that 1. the tetrafluoride solutions do not reduce erosion and erosion/abrasion of enamel and dentin in situ, 2. the tetrafluoride solutions are not equally or more effective than the AmF solution, 3. the fluoride solutions are not equally effective in reducing erosion and combined erosion/abrasion of the samples and 4. the fluoride solutions are not equally effective in preventing erosion or combined erosion/abrasion in enamel and dentin.

**Materials and methods**

Experimental design

This study was designed as crossover and double-blind study performed in eight phases (4 fluoride agents, 2 conditions: erosion (ERO) or erosion and abrasion (ERO + ABR)) of 3 days each, with a washout period of 3 days between the phases. Ten healthy adult volunteers (9 female, 1 male, aged 23-64 y) who fulfilled the inclusion criteria (physiological saliva flow rates: stimulated: >1 ml/min, unstimulated: >0.25 ml/min; good oral health: no frank cavities or significant gingivitis/ periodontitis) without violating the exclusion criteria (general/systemic illness, pregnancy or breastfeeding, use of fixed or removable orthodontic appliances) were enrolled following CONSORT guidelines. The sample size of 10 participants followed the sample size of previously performed in situ studies$^{14,15}$ and was calculated with a 95% level considering the desired precision of d = 0.1 (enamel) or d = 1 (dentin), respectively.

Ethic approval for the study was granted by the local Ethics Committee (No 07/11). Participants received written instructions and gave their written consent.

In each phase, the volunteers wore an acrylic intraoral appliance with 2 enamel and 2 dentin samples. One enamel and one dentin sample were treated once with TiF$_4$, ZrF$_4$, HfF$_4$ or AmF, while the controls remained unfluoridated. All samples were subjected either to extraoral erosion with a softdrink (Sprite®, Coca-Cola, Switzerland, 4 times/day, each 90 s) or to erosion followed by abrasion (2 times/day, 30 s/sample). Enamel and dentin loss was analysed profilometrically after 3 experimental days.
Preparation of enamel and dentin samples

Cylindrical enamel and dentin specimens (3 mm in diameter, in total 120 enamel and 120 dentin specimens) were obtained 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 moulds of a ceramic disc (Degussit, Friatec/Degussa, Düsseldorf, Germany) and fixed with composite material (Tetric EvoFlow, Ivoclar Vivadent, Schaan, Liechtenstein). Subsequently, enamel and dentin surfaces were ground flat and polished with water-cooled carborundum discs (1200, 2400 and 4000 grit, Water Proof Silicon carbide Paper, Stuers, Erkrat, Germany) thereby removing approximately 200 µm of the outermost layer as verified with a micrometer (Digimatic, Mitutoyo, Tokyo, Japan). The samples were sterilized by γ-radiation (12 kGy, Paul Scherrer Institute, Villigen, Switzerland).

Custom-made acrylic palatal devices were provided with 4 buccal recesses in the areas of left and right maxillary second premolars and first molars for attachment of the samples. The position of the two enamel and two dentine samples in the appliance was randomly determined for each volunteer and for each phase.

Fluoride Treatment

The appliances with specimens were inserted and worn for 2 h prior to the application of the fluorides to allow for the formation of a salivary pellicle, generally present on tooth surfaces in vivo.

Then, the respective solutions (1.55 % TiF₄, 2.09 % ZrF₄, 3.18 % HfF₄ or 13.43 % AmF), equimolar with respect to fluoride (0.5 M F) were applied once on one enamel and one dentin sample, while the remaining two samples were left unfluoridated and served as individual control. The tetrafluoride 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 pH of the solutions amounted to: TiF₄: 1.7, ZrF₄: 2.1 and HfF₄: 1.9. The AmF solution (GABA, Switzerland) presented a pH of 4.5.
(Metrom 827 pH Lab, Metrom, Herisau, Switzerland). The solutions were prepared freshly prior to application on the enamel or dentin specimens. Fifteen microliters of the respective solution were pipetted on the samples surface and remained undisturbed for 60 s. After that, the samples were rinsed with deionized water for 10 s (35 ml) and subjected to the oral cavity for 2 h before the first erosive challenge was performed.

The distribution of the fluoride and brushing treatments to the subjects in each phase was randomly determined. As well, the position of the samples in the appliance was randomized. Any cross contamination between fluoridated and non-fluoridated specimens could be excluded as they were fixed at a distance of at least 1 cm from each other in the appliance.\textsuperscript{16}

In situ experiment
The order of the treatments (TiF\textsubscript{4}, ZrF\textsubscript{4}, HfF\textsubscript{4} and AmF) and conditions (ERO or ERO + ABR), respectively, was randomly assigned to each participant. The appliances were worn day and night except for meals and personal oral hygiene. During meals and oral hygiene procedures, the samples were stored in wet gauze. The volunteers were instructed to brush their teeth twice daily in the morning and the evening with a fluoridated toothpaste (1400 ppm F, Elmex, GABA, Switzerland). After meals and drinks as well as after oral hygiene procedures, 10 min elapsed before reinsertion of the appliance. In each phase, all samples (fluoridated and unfluoridated/control) of the appliance were subjected to either ERO or ERO + ABR.

Erosion of the samples was done 4 times daily with each 3 h apart. Therefore, the appliance was immersed extraorally in 100 ml of Sprite (Coca-Cola, Switzerland) for 90 s and rinsed with tap water before reinsertion. Abrasion was performed twice a day, 90 min after the first and last erosive attack. The samples were brushed extraorally for 30 s each with a power toothbrush (Braun Oral-B Triumph, Braun Oral-B sensitive brushing head, Oral B, Switzerland) and 0.2 g toothpaste (1400 ppm F, Elmex, GABA, Switzerland). For standardized brushing force, the toothbrushes were fixed to a custom-made metal holder, which allowed for a 1.2 N contact pressure of the toothbrush head on the samples surface.
Profilometric analysis of enamel and dentin loss

Enamel or dentin loss, respectively, was analysed profilometrically using a mechanical profilometer (Perthometer S2, Mahr, Göttingen, Germany). Five profiles (distance between each other: 0.2 mm, length: 3.8 mm) were obtained from the center of each specimen by moving the diamond stylus across the samples surface and the reference areas (ceramic). This assessment was done at baseline and after completion of the respective 3-day phase. Identification marks (holes) made on the reference surface (ceramic) allowed accurate repositioning of the stylus allowing for exact superimposition of the respective baseline and final profile. The reproducibility of the measuring procedure was determined previously, and the coefficient of variation amounted to 1.36%.

The average depth of enamel or dentin loss relative to the baseline surface profiles was calculated using the software of the profilometer (Mahr Perthometer Concept 7.0, Mahr, Göttingen, Germany).

Statistical analysis

Enamel and dentin loss (µm) of fluoridated and control samples in the different groups was calculated for both conditions ERO and ERO + ABR. The data were statistically analysed by repeated measures Analysis of Variance (ANOVA) together with Greenhouse-Geisser correction separately for the fluoridated and control samples in eroded or eroded/abraded enamel or dentin, respectively. Paired t-tests were applied to compare fluoridated and control samples as well as eroded and eroded/abraded samples within each group. Additionally, enamel and dentin loss was calculated as percentage of the respective control to allow for comparison (paired t-test) of wear reduction in enamel and dentin and eroded and eroded/abraded samples, respectively. Finally, the reduction of enamel or dentin loss (% of control) was described by one sample analysis separately for each group. Possible carry-over effects were tested by the procedure pkcross of STATA version 10. The level of significance was set at p < 0.05.
Results

All participants satisfactorily finished the study. One dentin sample (HfF₄, control, ERO) got lost from the appliance during the experimental phase. Mean enamel and dentin loss (± standard deviation, µm) is displayed in Tables 1 and 2, respectively. Repeated measures ANOVA revealed no significant differences among fluoridated samples or among control specimens, respectively, for both conditions ERO and ERO + ABR and both substrates enamel and dentin. Generally, all fluoride groups showed significantly less wear compared to the controls, but enamel and dentin loss increased when the samples were additionally abraded (ERO + ABR) than eroded only (ERO).

In Fig.1, enamel and dentin loss is presented as percentage of the respective control (mean ± standard deviation). Enamel loss was significantly decreased in all groups compared to the control (100%). In dentin samples, fluoridation reduced ERO significantly in all groups, while loss due to ERO + ABR was decreased significantly only by AmF and TiF₄. Generally, the protective effect of the fluoride solutions was reduced under the condition ERO + ABR compared to ERO, but this effect was significant only for TiF₄ on enamel and ZrF₄ on dentin.

Pairwise comparisons between enamel and dentin samples of the same condition (ERO or ERO + ABR, respectively) revealed no differences within each fluoride group, indicating that the protective effect of the respective fluoride on enamel and dentin was not significantly different.

Generally, no carry-over effects could be observed (p = 0.85).

Discussion

The present in situ model was chosen to simulate the daily life situation in patients at risk for dental erosion as closely as possible. The erosive cycles were performed four times a day to imitate the frequent consumption of acidic drinks. Each erosion cycle was limited to 90 s as acids are neutralized and cleared on dental surfaces within few minutes.¹⁸ The samples were brushed twice daily as it mostly recommended by dentists for plaque control.¹⁹ Thereby, brushing was performed 90 min after the first and last erosive cycling, as it is known that a minimum of 60 min is needed to increase the abrasion resistance of erosively softened enamel and dentin to the range of sound dental hard tissues.²⁰,²¹ Brushing force was standardized to 1.2 N as the
brushing force of electrical toothbrushes might be somewhat lower than the values found clinically for manual toothbrushes.\textsuperscript{22,23} In contrast to the study of Hove et al.,\textsuperscript{9} the fluoride solutions were applied only once on pellicle-covered samples to simulate the professional application by a dentist. Thereby, the tetrafluorides were applied at concentrations distinctly below the concentrations of titanium, zirconium and hafnium compounds which might induce systemic side effects.\textsuperscript{24-27} AmF was chosen for comparison as it was shown to be more effective than sodium fluoride and equally effective to stannous fluoride (SnF\textsubscript{2}) to prevent enamel erosion at the same pH and concentration.\textsuperscript{28} The AmF solution presented a pH of 4.5 usually found in AmF-containing oral rinses.\textsuperscript{29} The tetrafluoride solutions were applied at their natural pH at it was shown previously that TiF\textsubscript{4} is less effective in preventing enamel and dentin erosion when the pH is buffered to a higher value (pH 3.5).\textsuperscript{30,31}

From previous in vitro studies it is known that an application time of 60 s is effective to induce the formation an acid-stable glaze-like layer (in case of TiF\textsubscript{4})\textsuperscript{30} or CaF\textsubscript{2}-like surface precipitates (in case of AmF),\textsuperscript{32} which are related to the erosion inhibiting properties of TiF\textsubscript{4} and AmF, respectively.

The results of the present study proved that tetrafluorides are able to reduce dental wear also under clinical conditions with frequent exposure to erosive and abrasive influences. This observation indicates that the surface precipitates formed after application of tetrafluorides might resist abrasive forces to some extent, not least as toothbrushing reduced the protective efficacy of all solutions only slightly (mostly not significant) compared to the groups which were eroded only. Only ZrF\textsubscript{4} and HfF\textsubscript{4} failed to reduce dentin wear due to erosion and abrasion significantly.

It might be speculated that the surface coating formed on dentin samples after application of HfF\textsubscript{4} and ZrF\textsubscript{4} might be more fragile than the precipitates formed after application of TiF\textsubscript{4}, although it was shown that zirconium applied on enamel in form of zirconium chloride lead to a relatively thick surface coating, while titanium chloride pretreatment induced a thinner surface layer.\textsuperscript{33}

However, it might be speculated that the protective effect of the tetrafluoride solutions might decrease under prolonged experimental conditions.\textsuperscript{34} In a previous experiment it was shown that the surface coating formed after application of TiF\textsubscript{4} is still present after 10 min of erosion, but can
not prevent the formation of a subsurface demineralisation below the glaze-like surface layer completely.\textsuperscript{30} In the case of AmF, it is speculated that the CaF\textsubscript{2}-like precipitations are dissolved\textsuperscript{35} and abraded\textsuperscript{36} with time, thus allowing for a direct contact of the acid with the sample surface. On the other hand, it might be assumed that a re-application or frequent application of the solutions might result in a higher degree of protection\textsuperscript{34,37} by leading to a surface layer more stable to erosion and abrasion. In a previous in situ study it was shown that enamel erosion was inhibited almost completely when TiF\textsubscript{4} was applied regularly.\textsuperscript{9} Thus, the frequent application of tetrafluorides would require preparations usable as mouth rinse or toothpaste which can be applied by the patients during oral hygiene. However, currently, the low pH of native tetrafluoride solutions (pH 1-2) has to be considered as major drawback, since it might cause adverse side effects on oral soft tissues during application and requires, therefore, the professional application by a dentist. In contrast, TiF\textsubscript{4} solutions with an acceptable pH for use in oral care products were shown to be ineffective in preventing dental erosion.\textsuperscript{30,31} Therefore, it seems an interesting approach to combine tetrafluorides with fluorides having stabilizing properties, such as amine fluoride, allowing TiF\textsubscript{4} to be also effective at a higher pH.\textsuperscript{34} However, the efficacy of a TiF\textsubscript{4}/AmF/ZnF\textsubscript{2} solution to prevent enamel erosion was proven only in vitro but not in situ as yet.\textsuperscript{34} Besides the pH value, the high fluoride concentration would also limit a daily application of the tetrafluoride agents.

In conclusion, the working hypotheses of the present study can be rejected as tetrafluorides were shown 1. to reduce erosion and erosion/abrasion of enamel and dentin, 2. to be equally effective to AmF, 3. to be equally effective in reducing erosion and combined erosion/abrasion and 4. to be equally effective in enamel and dentin.

\textbf{Acknowledgement}

We wish to thank the volunteers for participation in this study.
References


**Legends**

Tab. 1
Enamel loss (µm, mean ± standard deviation) in the fluoridated samples and the respective controls (not fluoridated) in the different groups. Significant differences between fluoridated and control samples within the conditions ERO or ERO + ABR, respectively, were marked by different capital letters, while differences between eroded and eroded/abraded fluoridated samples or controls, respectively, were marked by different small letters. Generally, no differences could be observed within the fluoridated and control groups for both conditions (ERO and ERO + ABR).

Tab. 2
Dentin loss (µm, mean ± standard deviation) in the fluoridated samples and the respective controls (not fluoridated) in the different groups. Significant differences between fluoridated and control samples within the conditions ERO or ERO + ABR, respectively, were marked by different capital letters, while differences between eroded and eroded and abraded fluoride samples or controls, respectively, were marked by different small letters. Generally, no differences could be observed within the fluoridated and control groups for both conditions (ERO and ERO + ABR).

Fig. 1
Enamel (a) and dentin (b) loss (% control, mean ± standard deviation) in the different groups. Wear was significantly decreased in all groups compared to the control (100%) except for HfF$_4$ and ZrF$_4$ in ERO + ABR (marked by *). The protective effect of the fluoride solution was decreased by additional abrasion of the samples (ERO + ABR) compared to ERO only, but this effect was significant only for TiF$_4$ on enamel and ZrF$_4$ on dentin (marked by #).
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<td>control</td>
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<td>AmF</td>
<td>0.4 ± 0.2&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>0.8 ± 0.2&lt;sup&gt;B,a&lt;/sup&gt;</td>
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<tr>
<td>TiF&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.5 ± 0.2&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>1.0 ± 0.4&lt;sup&gt;B,a&lt;/sup&gt;</td>
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<tr>
<td>HfF&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.5 ± 0.2&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>0.9 ± 0.3&lt;sup&gt;B,a&lt;/sup&gt;</td>
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<tr>
<td>ZrF&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.5 ± 0.2&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>0.8 ± 0.3&lt;sup&gt;B,a&lt;/sup&gt;</td>
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Tab. 2
Fig. 1