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Abstract

OBJECTIVE: This study aimed to analyse and compare the protective effect of buffered (pH 3.5) and native (pH 1.2) TiF(4) in comparison to NaF solutions of same pH on dentin erosion. DESIGN: Bovine samples were pretreated with 1.50% TiF(4) or 2.02% NaF (both 0.48M F) solutions, each with a pH of 1.2 and 3.5. The control group received no fluoride pretreatment. Ten samples in each group were eroded with HCl (pH 2.6) for 10x60s. Erosion was analysed by determination of calcium release into the acid. Additionally, the surface and the elemental surface composition were examined by scanning electron microscopy (two samples in each group) and X-ray energy-dispersive spectroscopy in fluoridated but not eroded samples (six samples in each group). Cumulative calcium release (nmol/mm²) was statistically analysed by repeated measures ANOVA and one-way ANOVA at t=10min. RESULTS: TiF(4) and NaF at pH 1.2 decreased calcium release significantly, while TiF(4) and NaF at pH 3.5 were not effective. Samples treated with TiF(4) at pH 1.2 showed a significant increase of Ti, while NaF pretreatment increased F concentration significantly. TiF(4) at pH 1.2 led to the formation of globular precipitates occluding dentinal tubules, which could not be observed on samples treated with TiF(4) at pH 3.5. NaF at pH 1.2 but not at pH 3.5 induced the formation of surface precipitates covering dentinal tubules. CONCLUSION: Dentin erosion can be significantly reduced by TiF(4) and NaF at pH 1.2, but not at pH 3.5.
TiF$_4$ and NaF at pH 1.2 but not at pH 3.5 are able to reduce dentin erosion

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Results: TiF₄ and NaF at pH 1.2 decreased calcium release significantly, while TiF₄ and NaF at pH 3.5 were not effective. Samples treated with TiF₄ at pH 1.2 showed a significant increase of Ti, while NaF pretreatment increased F concentration significantly. TiF₄ at pH 1.2 led to the formation of globular precipitates occluding dentinal tubules, which could not be observed on samples treated with TiF₄ at pH 3.5. NaF at pH 1.2 but not at pH 3.5 induced the formation of surface precipitates covering dentinal tubules.

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Key words
Dentin, erosion, fluoride, calcium, scanning electron microscopy
Introduction

The caries-preventive effect of titanium tetrafluoride (TiF$_4$) was shown more than 30 years ago.$^1$ Since then, numerous studies demonstrated that TiF$_4$ is able to reduce carious demineralisation in vitro$^2,3$ and to prevent the development of carious lesions in situ and in vivo.$^4,5$ The protective action of TiF$_4$ is mainly attributed to the formation of a glaze-like surface coating, which is assumed to be composed of titanium oxide$^6,7$ or of organometallic complexes.$^8$ TiF$_4$ might also lead to an increased fluoride uptake and, thus, reduce demineralisation chemically$^9,10$. The efficacy of TiF$_4$ to reduce carious demineralisation suggested that it might be also an effective agent in preventing erosive dental loss. In several recent in vitro$^3,11-13$ and few in situ studies$^{14,15}$ it was shown that TiF$_4$ reduced dental erosion significantly and was more effective than sodium, amine or stannous fluoride.

However, previous studies mainly concentrated on the erosion-inhibiting effect of TiF$_4$ on enamel, but only two studies evaluated the impact of TiF$_4$ solutions on dentin erosion as yet.$^{12,16}$ Although these studies found that TiF$_4$ was more effective in reducing dentin erosion than NaF$^{16}$ or AmF$^{12}$, the lack of data requires further studies dealing with the impact of TiF$_4$ on dentin erosion.

With regard to a potential implementation of TiF$_4$ as clinical product, some safety issues need to be answered prior to the patients’ self-application of TiF$_4$. Usually, TiF$_4$ solutions and varnishes present a very low pH (pH 1-2). Although this low pH might enhance the depth of penetration of fluoride ions, it might also induce adverse side effects on soft tissues (gingival or oral mucosa) during application. Sen et al.$^{17}$ showed that titanium tetrafluoride and acidulated phosphate fluoride (both pH 1.35) appeared to be more cytotoxic than sodium fluoride (pH 8.45) on L929 fibroblasts. Thereby, the low pH was considered as main factor causing the higher toxicity.
Thus, it would be desirable to use TiF$_4$ at higher pH, which is as effective as TiF$_4$ at low pH. In a previous investigation, the efficacy of TiF$_4$ at pH 1.2 and 3.5 on the prevention of enamel erosion was analysed. Thereby, it was shown that TiF$_4$ at pH 3.5 neither was effective in reducing erosive demineralisation nor induced the formation of a glaze-like surface layer.$^{18}$

With regard to the above mentioned considerations, this study aimed to analyse the efficacy of native (pH 1.2) and buffered (pH 3.5) TiF$_4$ solutions on dentin erosion and to compare the effects with NaF solutions of 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 same pH were equally effective.

**Material and Methods**

Sample preparation

Ninety cylindric dentin samples (3 mm in diameter) were prepared with a hollow drill from the labial root surface of ninety freshly extracted, non-damaged bovine incisors. The samples were embedded in acrylic resin (Paladur, Heraeus Kulzer, Germany) and ground flat and polished with water-cooled carborundum discs (1200, 2400 and 4000-grit, Water Proof 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 evident on the sample surfaces were selected for the study. The samples were randomly assigned to 5 groups. In each group, 10 samples were randomly selected for the erosion experiment, while 6 samples were used for X-ray energy-dispersive spectroscopy (EDS) and 2 samples were left for examination by Scanning Electron Microscopy (SEM). Prior to the experiment, the smear layer of all dentin samples was removed by application of 17% EDTA (pH: 8.0) for 60s. After that, the samples were rinsed with distilled water for 15s.
Fluoride solutions

The 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, USA) with 100 ml ultra pure 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 pH 3.5 by adding 45g H$_3$PO$_4$/100 ml and 12.6 g 5M H$_3$PO$_4$/100 ml, respectively. The pH of the solutions was measured by a pH electrode (Metrom 827 pH Lab, Metrom, Herisau, Switzerland). The solutions were prepared freshly prior to application on the dentin specimens.

Erosion experiment

Ten microliters of the respective fluoride solutions (1.50 % TiF$_4$, pH 1.2 and 3.5; 2.02 % NaF, pH 1.2 and 3.5) were pipetted on the samples 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.

Then, ten samples of each group were subjected to erosive treatment with hydrochloric acid (pH 2.6, 2.5 mmol/L) for 10 x 60s in sequence at room temperature. Each sample was stored for 60 s in 1 ml of HCl in an Eppendorf tube, which was gently shaken (60x/min) during sample incubation. After 60 s erosion, the samples were removed, rinsed with distilled water and placed in a new Eppendorf tube.

Determination of calcium loss

Calcium dissolved of the dentin samples during erosion was analysed by continuum source atomic absorption spectroscopy (ContrAA 300, Analytik Jena AG, Jena, Germany,
air/acytene flame) at 422.7 nm. The amount of calcium (µmol/mm²) released into the acid was determined in each 1 min acid fraction. The spectrometer was calibrated by calcium standard solutions. As phosphate might depress the sensitivity for calcium, 0.25% strontium chloride was added to the sample solutions to control these interferences.

X-ray energy-dispersive spectroscopy and Scanning Electron Microscopy
The Ti and F surface composition of the fluoridated specimens and the control samples was obtained by EDS and SEM (SUPRA 50VP and Genesis, Carl Zeiss NTS GmbH, Oberkochen, Germany). Eight dentin samples of each group were not subjected to erosion and were desiccated for 4 weeks in blue silica gel in a vacuum evaporator directly after treatment with the respective fluoride solution. EDS measurement was performed in six specimens of each group. Therefore, a defined area of 200 x 200 µm was measured in secondary electron mode (15 kV, 100 s). The weight percentage of the elements were analysed stoichiometrically. For SEM examination, two samples of each group were examined at 1 kV.

Statistical analysis
Cumulative calcium release was analysed by repeated measures analysis of variance (ANOVA) followed by Scheffe’s and Dunnett’s post-hoc tests. Moreover, cumulative calcium release at t = 10 min was analysed by one-way ANOVA followed by Scheffe’s and Dunnett’s post-hoc tests. The EDS data were analysed by one-way ANOVA and Scheffe’s post-hoc tests separately for each element. The level of significance was set at p < 0.05.

Results
Mean cumulative calcium loss (nmol/mm²) after 1 to 10 min of erosion is presented in Figure 1. Repeated measures ANOVA showed significant differences among the groups over time. TiF₄ and NaF at pH 1.2 reduced calcium release significantly compared to TiF₄ and NaF at
pH 3.5. While TiF₄ and NaF at pH 1.2 were equally effective, TiF₄ and NaF at pH 3.5 were significantly different. Also when considering t = 10 min only, cumulative calcium release (control: 101.4 ± 11.1 nmmol/mm²) was significantly reduced by TiF₄ (63.5 ± 12.0 nmmol/mm²) and NaF (67.6 ± 12.8 nmmol/mm²) at pH 1.2, but not by TiF₄ (115.2 ± 12.2 nmmol/mm²) and NaF (95.4 ± 19.3 nmmol/mm²) at pH 3.5.

The titanium and fluoride surface composition of the different groups is presented in Table 1. TiF₄ at pH 1.2 led to a significant increase of Ti compared to all other groups. Elemental surface composition in samples treated with TiF₄ at pH 3.5 was not significantly different from the control. The application of NaF led to a significant increase of F, which was distinctly higher for the solution at pH 1.2 than for the solution at pH 3.5.

The SEM images of the fluoridated samples are shown in Fig 2a-e. Pretreatment with TiF₄ at pH 1.2 (Fig. 2a) led to the formation of a globular surface coating occluding also the dentinal tubules. This globular surface coating was not observed on samples pretreated with TiF₄ at pH 3.5 (Fig 2b). The NaF solution at pH 1.2 (Fig. 2c) but not at pH 3.5 (Fig 2d) induced the formation of precipitates, which covered the dentinal tubules. Samples treated with NaF at pH 3.5 were free from precipitates and appeared similar to samples of the control group (Fig 2e).

**Discussion**

As recently shown for the efficacy of TiF₄ to prevent enamel erosion, the present study demonstrated that buffered TiF₄ (pH 3.5) was not effective in reducing dentin erosion. In contrast, TiF₄ at pH 1.2 reduced calcium loss significantly indicating that the efficacy of TiF₄ to prevent dental erosion is strongly associated to the native pH of the 1.50% TiF₄ solution.

In the present study, the samples treated with TiF₄ at pH 1.2 exhibited a globular titanium-rich surface layer. This surface coating might offer a mechanical protection of the underlying dentin and might be composed of organometallic complexes of titanium and the organic matrix of dentin. Alternatively, it is assumed that titanium reacts with oxygen (from water or
phosphate bound oxygen) or phosphate forming TiO₂ or titanium phosphate compounds, respectively.⁷,²³ These reactions seem to be favoured by a low pH of the TiF₄ solution²³ and might explain the absence of any surface granules on samples treated with TiF₄ at pH 3.5. In a previous study²² it was shown that the appearance of the surface coating is highly influenced by the presence of the smear layer. While a granular surface layer is formed on intertubular and intratubular dentin of smear layer-free samples (as in the present study), a modified smear layer and a massive structure was observed when TiF₄ is applied on smear layer-covered dentin.²² The hypothesis that the formation of the surface layer is dependent on the organic matrix is confirmed by Mundorff et al.⁸, who showed that the formation of a glaze-like layer after TiF₄ application was distinctly decreased on organic reduced enamel. However, considering the application of TiF₄ in the clinical situation, it is of higher relevance to evaluate the impact of the salivary pellicle which is usually present on tooth surfaces. In a previous study it was shown that the capacity of TiF₄ to protect dentin against erosive calcium loss was better in pellicle-covered than in pellicle-free samples.¹²

As shown previously, the surface concentration of titanium was significantly increased after application of TiF₄ at pH 1.2.²⁴,²⁵ The fluoride surface concentration was also increased compared to the control, although not significantly, and was in the range found previously for TiF₄-treated dentin.²⁴ Thus, the surface coating might act only partly as reservoir for fluoride ions which might retard acid dissolution chemically. Considering the high amount of fluoride found after application of NaF, it might be speculated whether TiF₄ treatment promoted a deeper penetration of fluoride²⁴, while NaF could favour a superficial fluoride bond in the outermost dentin surface.

In contrast to the TiF₄ solution at pH 1.2, the TiF₄ solution at pH 3.5 failed to reduce calcium release. In accordance to the absent surface layer, the titanium surface concentration was only slightly, but not significantly increased compared to the control. These results indicate that the
protective capability of TiF\(_4\), in particular the formation of the granular surface coating, might be highly dependent on the pH of the solution.

The erosion-preventing effect of NaF is usually associated to the formation of CaF\(_2\)-like precipitates on the surface.\(^{26}\) Both groups treated with NaF showed a distinctly increased surface fluoride concentration, but did not present the globular structure of loosely-bound fluoride usually found on dentin treated with acidulated NaF.\(^{27-29}\) While samples treated with NaF at pH 3.5 did not present visible surface precipitates even at a higher magnification (data not shown), it might be hypothesized whether the fluoride precipitates formed after application of NaF at pH 1.2 are different from the typically found globular structure. Alternatively, it might be assumed that fluoride is structurally bound in the outermost dentin surface. However, the absence of any surface precipitates on samples treated with NaF at pH 3.5 might account for the lacking protective effect on calcium release.

The protective effect of TiF\(_4\) and NaF at pH 1.2 is in accordance to the study of Schlueter et al.\(^{16}\) In this study it was shown that both solutions (NaF and TiF\(_4\) at pH 1.2) were equally effective in reducing dentin erosion in a 5 day de- and remineralisation cycling. The fact that dentin erosion was reduced more efficiently than in the present study to approximately 25-30\% of the control might be explained by the duration and frequency of application of the solutions (5 min daily).\(^ {16}\)

In the present experiment, the fluoride solutions were applied only once for 60 s as done in previous studies\(^ {30,31}\) to simulate a realistic application time under clinical conditions. Although it was shown recently that even a single application of a TiF\(_4\) varnish\(^ {30}\) or a TiF derivative\(^ {32}\) was effective to prevent enamel demineralisation in pH-cycling models, further studies have to evaluate whether the surface precipitates formed after application of TiF\(_4\) at pH 1.2 on dentin are also stable over time, thus providing a long-lasting preventive effect against erosion.
Bovine dentin is widely used in erosion research as it presents similar chemical and mechanical properties to human dentin. Bovine teeth derived of similar genetic lineage and dietary environment might show a higher homogeneity of mineral composition than different human teeth, which are collected from various donators with diverse dietary or fluoride supplementation. However, for extrapolation of in vitro data to the clinical situation it should be taken into account that the susceptibility of human dentin to erosion is slightly higher compared to bovine dentin.\textsuperscript{33} Moreover, it has to be taken into consideration that the efficacy of TiF\textsubscript{4} to prevent erosion might be also slightly different between human and bovine samples, as indicated by the study of Hove et al.\textsuperscript{34} performed on human and bovine enamel. From the results of the present study it can be concluded that the efficacy of TiF\textsubscript{4} to prevent dentin erosion is associated to the low pH of the native solution. Thus, the first working hypothesis that TiF\textsubscript{4} at pH 1.2 and 3.5 are equally effective in reducing dentin erosion is rejected, while the second hypothesis that TiF\textsubscript{4} and NaF of same pH are equally effective is accepted.

\textbf{Acknowledgement}

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Figure 1

Cumulative calcium release (nmol/mm²) in the different groups after 1 to 10 min erosion: x = control, ■ = TiF₄, pH 1.2; □ = TiF₄, pH 3.5, ● = NaF, pH 1.2; ○ = NaF, pH 3.5. Groups marked with the same letter were not statistically different at t = 10 min.
Figure 2

SEM images of the different groups at 40,000 magnification
While different surface precipitates could be observed in samples treated with TiF$_4$ (a) and NaF (c) at pH 1.2, samples treated with TiF$_4$ (b) and NaF at pH 3.5 (d) did not exhibit surface precipitation and appeared similar to the control (e).
<table>
<thead>
<tr>
<th></th>
<th>Ti</th>
<th>F</th>
</tr>
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<td>Control</td>
<td>0.0 ± 0.0\textsuperscript{a}</td>
<td>0.1 ± 0.1\textsuperscript{a}</td>
</tr>
<tr>
<td>TiF\textsubscript{4}, pH 1.2</td>
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<td>1.1 ± 0.2\textsuperscript{a}</td>
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<td>0.6 ± 0.1\textsuperscript{a}</td>
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<td>NaF, pH 1.2</td>
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<td>17.1 ± 2.7\textsuperscript{b}</td>
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<td>NaF, pH 3.5</td>
<td>0.1 ± 0.1\textsuperscript{a}</td>
<td>10.0 ± 3.1\textsuperscript{c}</td>
</tr>
</tbody>
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Table 1

Titanium and fluoride surface composition (%) in the different groups.

Within each element, groups marked with the same letter were not statistically different.