The effect of an experimental 4% TiF4 varnish compared to NaF varnishes and 4% TiF4 solution on dental erosion in vitro

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Abstract

This in vitro study assessed the effect of an experimental 4% TiF₄ varnish compared to commercial NaF and NaF/CaF₂ varnishes and 4% TiF₄ solution on enamel erosion. For this, 72 bovine enamel specimens were randomly allocated to the following treatments: NaF varnish (2.26% F), NaF/CaF₂ varnish (5.63% F), 4% TiF₄ varnish (2.45% F), F-free placebo varnish, 4% TiF₄ solution (2.45% F) and control (not treated). The varnishes were applied in a thin layer and removed after 6 h. The solution was applied to the enamel surface for 1 min. Then, the specimens were alternately de- and remineralized (6 times/day) in an artificial mouth for 5 days at 37 degrees C. Demineralization was performed with the beverage Sprite (1 min, 3 ml/min) and remineralization with artificial saliva (day: 59 min, 0.5 ml/min; during the night: 0.1 ml/min). The mean daily increment of erosion and the cumulative erosion data were tested using ANOVA and ANCOVA, respectively, followed by Tukey's test (alpha = 0.05). The mean daily erosion increments and cumulative erosion (micrometers) were significantly less for the TiF₄ varnish (0.30 +/- 0.11/0.65 +/- 0.75) than for the NaF varnish (0.58 +/- 0.11/1.47 +/- 1.07) or the NaF/CaF₂ varnish (0.62 +/- 0.10/1.68 +/- 1.17), which in turn showed significantly less erosion than the placebo varnish (0.78 +/- 0.12/2.05 +/- 1.43), TiF₄ solution (0.86 +/- 0.11/2.05 +/- 1.49) and control (0.77 +/- 0.16/2.06 +/- 1.49). In conclusion, the TiF₄ varnish seems to be a promising treatment to reduce enamel loss under mild erosive conditions.
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This in vitro study assessed the effect of an experimental 4%TiF₄ varnish compared to commercial NaF and NaF/CaF₂ varnishes and 4%TiF₄ solution on enamel erosion. For this, 72 bovine enamel specimens were randomly allocated to the following treatments: NaF varnish (2.26%F), NaF/CaF₂ varnish (5.63%F), 4% TiF₄ varnish (2.45% F), F-free placebo varnish, 4% TiF₄ solution (2.45%F) and control (not treated). The varnishes were applied in a thin layer and removed after 6 h. The solution was applied to the enamel surface for 1 min. Then, the specimens were alternately de- and re-mineralized (6 times/day) in an artificial mouth for 5 days at 37°C. Demineralization was performed with the beverage Sprite (1 min, 3 mL/min) and remineralization with artificial saliva (day: 59 min, 0.5 mL/min, during night: 0.1 mL/min). The mean daily increment of erosion and the cumulative erosion data were tested using ANOVA and ANCOVA, respectively, followed by Tukey’s test (α = 0.05). The mean daily erosion increments and cumulative erosion (µm) were significantly less for the TiF₄ varnish (0.30 ± 0.11/0.65 ± 0.75) than for NaF varnish (0.58 ± 0.11/1.47 ± 1.07) or NaF/CaF₂ varnish (0.62 ± 0.10/1.68 ± 1.17), which in turn showed significantly less erosion than placebo varnish (0.78 ± 0.12/2.05 ± 1.43), TiF₄ solution (0.86 ± 0.11/ 2.05 ± 1.49) and control (0.77 ± 0.16/2.06 ± 1.49). In conclusion, the TiF₄ varnish seems to be a promising treatment to reduce enamel loss under mild erosive conditions.
Introduction

Dental erosion is the loss of tooth tissue by chemical processes not involving bacteria [Imfeld, 1996; Moss, 1998; Lussi, 2006]. Although a multitude of factors seem to be involved in this process, the most important factors are dietary acids [Lussi et al., 2004; Lussi and Jaeggi, 2006] and intrinsic acids from the stomach [Scheutzel, 1996; Bartlett, 2006]. Currently, the increased consumption of acidic foods and soft drinks is becoming an important factor for the development of erosive wear [Lussi et al., 2004; Lussi, 2006].

As it is difficult to control possible etiological factors, many strategies have been developed for the prevention of erosion, such as the topical application of fluoride. Although the preventive action of fluoride on dental caries is well known [ten Cate, 1997], its role in erosion is still controversially discussed [Larsen and Richards, 2002; Wiegand and Attin, 2003], since the deposited calcium fluoride-like material from topical fluoride application is supposed to dissolve readily in most acidic drinks [Ganss et al., 2007].

The formation of the CaF$_2$-like layer and its protective effect on demineralization depend on the pH, F concentration and type of F salt of the agent [Saxegaard and Rölla 1988]. Highly concentrated fluoride applications, such as oral rinses, gels or varnishes, have been demonstrated to increase abrasion resistance and decrease the development of enamel erosion in vitro and in situ [Ganss et al., 2004; Vieira et al. 2006]. Most studies focusing on the preventive effect of fluoride on erosion used fluoride compounds which have been used over years in caries prevention, such as NaF, AmF, SnF$_2$ or acidulated phosphate fluoride (APF). More recently, other agents, such as titanium tetrafluoride (TiF$_4$), have been investigated for erosion prevention [Tveit
et al., 1983; Büyükyılmaz et al., 1997; van Rijkom et al., 2003; Vieira et al., 2005, 2006; Hove et al., 2006, 2007; Schlueter et al., 2007; Magalhães et al., 2007a).

Several in vitro studies have shown an inhibitory effect of TiF$_4$ solution on dental erosion [Büyükyılmaz et al., 1997; van Rijkom et al., 2003; Hove et al., 2006, 2007; Schlueter et al., 2007], which is attributed not only to the effect of fluoride, but also to the action of titanium [Buyukyilmaz et al., 1997; Tezel et al., 2002]. It is speculated that 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 [Mundorff et al., 1972; Büyükyılmaz et al., 1997; Tezel et al., 2002; Ribeiro et al., 2006]. Moreover, it is suggested that titanium interacts with the enamel surface, because of the low pH of the agent, thus leading to an increased fluoride uptake by enamel [Mundorff et al., 1972; Gu et al., 1996]. However, other studies did not find a protective effect of TiF$_4$ against erosion or combined erosion and abrasion [Vieira et al., 2005, 2006; Magalhães et al., 2007a].

Because of their capacity to adhere to the tooth surface and create a calcium fluoride reservoir [Sorvari et al., 1994; Vieira et al., 2007], fluoride varnishes might be more effective than solutions and gels in the prevention of erosive defects [Sorvari et al., 1994; Vieira et al., 2007]. Thus, the use of an experimental TiF$_4$ varnish for the prevention of erosive wear has been proposed. In a previous study, TiF$_4$ varnish did not reduce enamel loss under severe erosive conditions [Magalhães et al., 2007b]. Thus, the present study aimed to compare the same TiF$_4$ varnish with a TiF$_4$ solution (the most investigated form of TiF$_4$ application) and commercial NaF and NaF/CaF$_2$
varnishes (as positive controls) in an in vitro model characterized by a milder erosive challenge.

Material and Methods

Specimen preparation

Seventy-two crowns of bovine incisors were embedded in acrylic resin cylinders (Paladur, Heraeus Kulzer, Wehrheim, Germany), and the labial surfaces were ground flat and polished with water-cooled carborundum paper (500, 800, 1200, 2400 and 4000 grit, waterproof silicon carbide paper; Struers, Erkrat, Germany), approximately 200 µm of the outer enamel being removed. Surface hardness of enamel specimens was used as a criterion for stratified allocation of the specimens among 6 groups of 12. Prior to the experiment, baseline scans were obtained from the specimens with a contact profilometer (Mahr Perthometer, Göttingen, Germany). Reference areas on the polished enamel surface were covered with parallel strips of adhesive tape (Tesa, Beiersdorf, Hamburg, Germany), 1.5 mm apart. After preparation, the specimens were stored in water until used for the experiment to avoid dehydration.

Fluoride pre-treatment

Prior to acid exposure, the specimens were pre-treated as follows: (1) NaF varnish (2.26% F, pH 4.5; Duraphat, Colgate-Brazil); (2) NaF/CaF$_2$ varnish NaF, (2.71% F as NaF, 2.92% F as CaF$_2$, pH 8.0; Duofluorid, FGM-Brazil); (3) 4% TiF$_4$ varnish (2.45% F, pH 1.0; FGM-Brazil); (4) F-free placebo varnish (pH
5.0; FGM-Brazil); (5) 4% TiF$_4$ solution (2.45% F, pH 1.0); (6) no treatment (control). While Duofluorid is transparent; the other varnishes are yellow. According to the manufacturers, the Brazilian varnishes (FGM) contain colophonium, synthetic resin, thickening polymer, essence, artificial sweetener and ethanol, while Duraphat contains 2.26% NaF, 33.1% alcohol, natural resins (colophonium, mastix, shellac), wax, saccharine and flavor. All varnishes had a soft consistency. To prepare the 4% TiF$_4$ solution, solid TiF$_4$ (Aldrich Chemical Company, Milwaukee, WI, USA) was dissolved in deionized water. The pH of all solution/varnishes was measured by indicator paper (± 0.5 units).

The TiF$_4$ solution was applied with a microbrush and left on the surface undisturbed until the surface appeared dry. Additional drops were applied in the same manner over the course of 1 min [van Rijkom et al., 2003]. Excess solution was removed from the surface by a cotton roll and the specimens were stored in artificial saliva [Klimek et al., 1982].

The varnishes were applied in a thin layer using a microbrush and the specimens were stored in artificial saliva. After 6 h, the varnishes were carefully removed using acetone and a scalpel blade, taking care to avoid touching of the enamel surface. Completeness of removal layer was checked microscopically. Prior to cycling, surface loss due to application of the varnishes or solution was determined by profilometry.

**pH-cycling**

For pH cycling, the specimens were mounted in an artificial mouth [Attin et al., 2003] for 5 days allowing for alternating de- and remineralization treatment. The artificial mouth consisted of 12 chambers, heated to 37°C, and
was equipped with two automatic multichannel pumps (IPC/IPC-N Kassetten-Schlauchpumpen, Ismatec SA, Glattbrugg-Zürich, Switzerland). Temperature and pumps were controlled by a computer and specially written software. Only one specimen was placed in each chamber. The specimens were randomly distributed to the 12 chambers in each pH cycle, 2 specimens from each group being randomly selected and submitted to the 5-day cycle in the artificial mouth.

The artificial mouth was programmed so that the specimens were rinsed with Sprite (3 mL/min, Coca-Cola Company, USA, pH 2.6) 6 times a day for 1 min each. Between erosive challenges, the specimens were rinsed continuously with artificial saliva [Klimek et al., 1982] (0.5 mL/min, 59 min). The composition of the artificial saliva was: 0.2 mM glucose, 9.9 mM NaCl, 1.5 mM CaCl₂.2H₂O, 3 mM NH₄Cl, 17 mM KCl, 2 mM NaSCN, 2.4 mM K₂HPO₄, 3.3 mM urea, 2.4 mM NaH₂PO₄ and ascorbic acid. After 6 de- and re-mineralization treatments, enamel surface loss was determined by profilometry, and the specimens were rinsed with artificial saliva overnight (0.1 mL/min, 18 h).

**Profilometry**

Surface profiles of the enamel specimens were obtained with a contact profilometer (Mahr Perthometer, Göttingen, Germany) prior to the experiment, after the respective fluoride pre-treatment and after every 5 daily de- and re-mineralization cycles. The reference areas, which remained protected by tape during fluoride pre-treatment and during the entire daily de- and remineralization cycling, were marked with a scalpel blade on the outer surface to allow for exact reposition of the tape. Prior to the experiment, five equidistant baseline surface scans of each specimen were performed. For determination of enamel loss the
tape was removed and five profiles were recorded at exactly the same sites as for baseline measurement. For this, the enamel specimens were provided with identification marks, which allowed the stylus to be re-positioned accurately at each measurement. The profile scans were performed in the centre of each specimen at intervals of 250 µm. Pre- and post-treatment scans were superimposed and the average depth of the area under curve in the eroded area was calculated with specially designed software. The results of the five scans at each day were averaged for each specimen.

Scanning Electron Microscopy (SEM)

To show the interaction between the TiF₄ varnish and the solution with enamel, pairs of polished bovine enamel specimens (4 x 4 mm) were freshly treated with TiF₄ varnish, TiF₄ solution or remained untreated (control). For this, three specimens were obtained from each of two teeth, and one was allocated to each treatment group. The samples were treated for 1 min with the TiF₄ solution and for 6 h with TiF₄ varnish in exactly the same way as for the main experiment, the varnish again being removed after treatment using acetone and a scalpel blade. The two control samples remained untreated.

After treatment, the specimens were carefully dried with paper, coated with gold/palladium using sputter coating, dried by vacuum and examined with a scanning electron microscope (XL 30 FEG SEM; Philips, The Netherlands, with field emission gun at 20KV) in both secondary electron and backscattered electron modes. Elemental surface composition of the whole enamel surface was obtained using x-ray energy dispersive spectroscopy (EDS) using an ISIS 300 spectrometer (Link; Oxford, U.K.) coupled to the SEM. In the samples
treated with the TiF₄ varnish, the whole surface was analyzed, including the surface layer and the enamel below this layer. Determinations were made using quantitative analysis with ZAF correction.

Statistical analysis

To test whether the treatments caused any significant alteration of the enamel surface, the profiles obtained before and after application of the agent were compared within each group by paired t-tests. Mean daily erosion increments were calculated for each treatment. Linear regressions for cumulative erosion on number of treatments were calculated for each treatment (days 0-5; day 0 being the profile after treatment). The assumptions of equality of variances and normal distribution of data were checked for all the variables tested, using the Bartlett and Kolmogorov-Smirnov tests, respectively. Since the assumptions were satisfied, differences in the mean daily erosion increment were tested using one-way ANOVA and the cumulative erosion regressions were compared by ANCOVA. Tukey’s test was used for post-hoc pairwise testing. Graph Pad InStat version 3.0 for Windows, Graph Pad Software (San Diego, CA, USA) and Statistica v.5.1, StatSoft Inc. (Tulsa, USA) software were used for statistical analysis. The significance level was set at 5%.

Results

The application of the TiF₄ solution produced significant enamel loss (p < 0.001) when compared to the baseline profile after the polishing. In contrast, the TiF₄ varnish provoked a significant growth of the surface (p = 0.043). The other
groups did not present any significant difference compared to baseline profile after polishing (Table 1).

Figure 1 shows the linear regression lines and the cumulative erosion for the treatments at each day, considering the baseline after the application of the agents as day 0 (Days 0-5). ANCOVA of the linear regressions (days 0-5) showed that mean daily cumulative erosion (µm) was significantly less for the TiF₄ varnish (0.65 ± 0.75) than for NaF varnish (1.47 ± 1.07) or NaF/CaF₂ varnish (1.68 ± 1.17) which did not differ significantly from each other. However, both showed significantly less erosion (p < 0.001) than placebo varnish (2.05 ± 1.43), TiF₄ solution (2.05 ± 1.49) and control (2.06 ± 1.49), which did not differ significantly.

Table 1 shows the daily increments of enamel erosion. ANOVA showed that the mean daily increment (µm) was significantly less for TiF₄ varnish (0.30 ± 0.11) than for NaF varnish Duraphat (0.58 ± 0.11) or NaF/CaF₂ varnish (0.62 ± 0.10), both of which showed significantly less erosion (p < 0.001) than placebo varnish (0.78 ± 0.12), TiF₄ solution (0.86 ± 0.11) and control (0.77 ± 0.16), which did not differ significantly.

The SEM image of the untreated enamel sample presented a polished surface (Figure 2). Enamel treated with TiF₄ varnish presented a thin smooth surface layer in which microcracks were visible (Figure 3). In contrast, enamel treated with TiF₄ solution showed an etched surface (Figure 4). For TiF₄ solution treated samples, at low power a honeycomb appearance reflecting the prism structure was visible (not shown). Ti and F were not detectable at the untreated enamel surfaces, but enamel treated with TiF₄ varnish exhibited mean surface concentrations of 3.5 ± 1.0% Ti and 2.0 ± 0.80% F. Enamel treated with the TiF₄
solution showed mean surface concentrations of 0.96 ± 0.06% Ti and 1.75 ± 0.90% F.

**Discussion**

The conflicting results for TiF₄ in previous studies [Büyükýilmaz et al., 1997; van Rijkom et al., 2003; Vieira et al., 2005, 2006; Hove et al., 2006, 2007; Schlueter et al., 2007; Magalhães et al., 2007a] can be explained by the different protocol used, as well as the different response variables analyzed.

In order to improve the erosion protective efficacy of TiF₄ by increasing the adherence to dental hard tissues, the TiF₄ was applied in the present study in the form of a varnish. The varnishes and the solution were applied only once in order to simulate the clinical situation with a single professional application by a dentist. In contrast to most of the studies showing a protective effect of fluoride varnishes against dental erosion [Vieira et al., 2005, 2006, 2007], the varnishes were completely removed after 6 h in the present study. This was done to focus on the chemical effect of the varnishes (NaF or CaF₂ versus TiF₄) rather than on the mechanical protection. Thus, the varnishes were removed to simulate the clinical situation in which the varnishes might be removed by toothbrushing or mastication after some hours. This fact is especially important for eroded surfaces, which are usually more susceptible to mechanical forces.

A mild erosive challenge of the specimens was conducted during 5 days to simulate a frequent acid contact in the in vivo situation. Enamel erosion was assessed by contact profilometry. It must be remembered that the stylus might
be able to scratch the acid softened surface [Barbour and Rees, 2004] but this would occur in all the groups, so should not bias the results.

Regarding the positive controls, most previous studies tested the effect of commercial varnishes containing NaF, and found a reduction of erosion in vitro and in situ [Sorvari et al., 1994; Vieira et al., 2005, 2006, 2007]. In the present study, a NaF varnish and a NaF/CaF$_2$ varnish reduces enamel loss significantly when compared to the placebo varnish, TiF$_4$ solution and control, and they did not differ from each other.

The experimental TiF$_4$ varnish showed the best protective effect on erosion in this mild 5-days erosive protocol. On the other hand, the TiF$_4$ solution did not provide a preventive effect against enamel erosion during 5 days of de- and re-mineralisation. The SEM and EDS results might help to explain this difference in behavior of the TiF$_4$ when employed as a varnish or as a solution. Enamel treated with TiF$_4$ solution showed an etched appearance which is consistent with the small profilometric loss after treatment observed both in this study (Table 1) and also by Magalhães et al. [2007a]. This contrasts with the layer produced by treatment with TiF$_4$ varnish, which probably provides a mechanical and chemical barrier, even though it has cracks [Tveit et al., 1983; Büyükyilmaz et al., 1997; Vieira et al., 2006; Hove et al., 2006]. Besides the layer on the enamel surface, TiF$_4$ varnish-treated enamel had a higher surface concentration of both Ti and F than the TiF$_4$ solution-treated surface. The different surface effects of TiF$_4$ formulations seem to be related to the better ability of the varnish to adhere to the tooth surface, which allows an increased contact time with the enamel and hence prolongs the reaction between the TiF$_4$ and enamel [Vieira et al., 2006, 2007]. The lack of erosion after TiF$_4$ varnish
(Table 1) suggests that the varnish might reduce the effects of the low pH on the enamel.

However, because TiF₄ varnish was more protective of enamel against erosion by 30 min treatment with Sprite than against erosion 240 min exposure to cola [Magalhães et al, 2007b], it is possible that the protective layer would be removed over time with erosive challenges. In the clinical situation this experimental varnish might be less effective in preventing dental erosion for a long time, especially in patients at high risk for erosion. The persistence of the protective effect of TiF₄ varnish and the frequency of application have to be evaluated in further studies, using experimental times higher than 5 days.

Since Vieira et al. [2005, 2006] did not find a protective effect of a TiF₄ solution on combined enamel erosion and abrasion; it would be interesting to test the efficacy of this experimental varnish against abrasive forces. Moreover, the effect of this varnish on human enamel should be evaluated. Hove et al. [2007] showed that the protective effect of a TiF₄ solution was better for bovine compared to human enamel, especially when a salivary pellicle was present on the surface.

Under the conditions of the present in vitro protocol, it can be concluded that TiF₄ varnish, but not TiF₄ solution, reduced erosive bovine enamel erosion. Moreover, TiF₄ varnish showed better results than two commercial NaF varnishes. However, more studies must be conducted in situ and in vivo, before TiF₄ varnish can be widely used in the clinical situation.

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References


Figure 1. The linear regression line and the cumulative erosion points for the treatments at each day, considering baseline (zero) as profile after the treatment. Open circle = control ($r^2=0.81$); open triangle = placebo varnish ($r^2=0.87$); open square = TiF$_4$ solution ($r^2=0.90$); solid diamond = Duraphat ($r^2=0.83$); solid square = Duofluorid ($r^2=0.82$); solid circle = TiF$_4$ varnish ($r^2=0.62$).
Figure 2. SEM image of untreated enamel (control).
Figure 3 - SEM images of enamel treated with 4% TiF$_4$ varnish, showing a continuous surface layer with microcracks.

Figure 4. SEM image of enamel treated with 4 % TiF$_4$ solution, showing an etched appearance.
Table 1. Daily increment of erosion (µm) for each experimental group. Negative numbers signify growth of the surface. Means ± SD

<table>
<thead>
<tr>
<th>Days Treatment</th>
<th>Duraphat</th>
<th>Duofluorid</th>
<th>TiF₄ varnish</th>
<th>Placebo varnish</th>
<th>TiF₄ solution</th>
<th>Control</th>
</tr>
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<tr>
<td></td>
<td>-0.01 ± 0.10</td>
<td>0.01 ± 0.10</td>
<td>-0.12 ± 0.18</td>
<td>0.02 ± 0.10</td>
<td>0.24 ± 0.14</td>
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<td>1</td>
<td>0.68 ± 0.27</td>
<td>0.68 ± 0.23</td>
<td>0.10 ± 0.25</td>
<td>0.91 ± 0.32</td>
<td>0.73 ± 0.17</td>
<td>0.90 ± 0.23</td>
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<td>2</td>
<td>0.59 ± 0.18</td>
<td>0.83 ± 0.27</td>
<td>0.27 ± 0.16</td>
<td>0.88 ± 0.21</td>
<td>0.78 ± 0.20</td>
<td>0.94 ± 0.34</td>
</tr>
<tr>
<td>3</td>
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<td>0.27 ± 0.13</td>
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