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Summary

Objective: This study aimed to compare the effects 0.5% and 1% sodium, amine and stannous fluoride at different pH on enamel erosion in vitro.

Methods: Bovine enamel samples were submitted to a cyclic de- and remineralisation for three days. Each day, the samples were exposed for 120 min to pooled human saliva and subsequently treated with one of the fluoride solutions for 3 min: amine fluoride (AmF, 0.5% and 1% F), sodium fluoride (NaF, 0.5% and 1% F), each at pH 3.9 and 7.0, and stannous fluoride (SnF₂, 0.5% and 1% F⁻), at pH: 3.9. Additionally, two groups were treated with fluoride-free placebo solutions (pH: 3.9 and 7.0) and one group served as control (no fluoridation). Ten specimens each group were inserted in a so-called artificial mouth and eroded six times daily with hydrochloric acid (pH 2.6) for 90 s each intermitted by exposure to artificial saliva (1h). After three days, enamel loss was analysed profilometrically and evaluated statistically by ANOVA.

Results: Only the acidic 0.5% and 1% SnF₂ and 1% AmF solutions were able to reduce erosive enamel loss significantly, while all other solutions and placebos did not differ significantly from the control. Between the acidic SnF₂ and the 1% AmF solutions no significant differences could be detected.

Conclusion: At the same concentrations, acidic SnF₂ and AmF may be more effective than NaF to protect enamel against erosion.
Introduction

One approach in the preventive management of dental erosion is the use of topical fluorides in form of toothpastes, solutions, gels and varnishes. The efficacy of different fluoride compounds to decrease the progression of the erosive process has been demonstrated in several studies.\textsuperscript{9,13} Thereby, most studies focused on fluoride compounds that have been used over years for caries prevention, such as sodium fluoride (NaF), stannous fluoride (SnF\textsubscript{2}) or amine fluoride (AmF). The protective anticaries effect of fluoride is mainly attributed to the formation of a CaF\textsubscript{2}-like layer on the tooth surface, which acts as a fluoride reservoir. During an acidic attack, fluoride released from the CaF\textsubscript{2}-deposit can be incorporated into the mineral by forming fluoroapatite or fluorohydroxyapatite resulting in a decreased susceptibility to further dissolution. A similar mode of action is assumed for the anti-erosive capability of fluorides. Additionally, the CaF\textsubscript{2}-layer might act as a mechanical barrier hampering the contact of the acid with the underlying enamel or as a mineral reservoir, which is attacked by the erosive challenge, thus leading to a buffering or depletion of hydrogen ions from the acid. The formation of the CaF\textsubscript{2}-layer depends on the pH and the concentration of the fluoride agent and the duration of application.\textsuperscript{17} As high concentrated fluoride agents or a prolonged application time might lead to a thicker and more stable CaF\textsubscript{2}-precipitate, an intensive fluoridation is considered as most effective for prevention of erosive enamel loss.\textsuperscript{4,13}

While most studies focused on the impact of the fluoride concentration and the pH-value of the fluoride agent on erosion, only few studies compared the efficacy of fluorides with respect to the fluoride compound. Thereby, SnF\textsubscript{2} containing toothpastes and solutions showed a better protective capability against erosion-like lesions than NaF agents.\textsuperscript{10,11,23,24} Ganss et al.\textsuperscript{5} found that erosive mineral loss was nearly completely inhibited by AmF/SnF\textsubscript{2} and SnF\textsubscript{2} solutions, while NaF solutions were less effective and AmF and AmF/NaF showed no significant effect (all solutions:
250 ppm F⁻). The results of this study suggest that not only the fluoride, but also the respective cation could influence the protective efficacy of the fluoride compound.

However, in most of these studies, the fluoride compounds were applied with different concentration of F,\textsuperscript{24} different pH\textsuperscript{10,11,23} or in different formulations (toothpaste, solution, gel or varnish),\textsuperscript{19} which makes the interpretation of the results regarding to the impact of the respective cations difficult. Moreover, in some in vitro experiments, the fluoride agents were not applied on pellicle-covered samples but on polished surfaces.\textsuperscript{5,19} This does not reflect the clinical situation very well, not least at it is known that the acquired pellicle might influence the retention of KOH-soluble fluoride in situ.\textsuperscript{6}

Thus, the present study aimed to compare the protective effect of different acidic (pH: 3.9) and neutral fluoride compounds with equimolar concentrations of fluoride (0.5% or 1% F⁻) on pellicle-covered enamel erosion. The null hypothesis tested was that the different fluoride solutions will not exhibit different protective potential on enamel erosion.

**Material and Methods**

**Enamel sample preparation**

Onehundred-thirty cylindric enamel samples (3 mm in diameter) were obtained from the labial surfaces of freshly extracted, non-damaged bovine incisors, which were stored in 0.9% NaCl solution until used. The teeth were extracted from cattles free of bovine spongiform encephalopathy. The samples were embedded in acrylic resin (Paladur, Heraeus Kulzer, Germany), and their labial 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 150 µm of the outermost layer as checked with a micrometer (Digimatic, Mitutoyo, Tokyo, Japan). The samples were randomly assigned to 13 groups with each 10 specimens.
**Fluoride solutions**

The experimental fluoride solutions were provided by GABA International, Münchenstein, Switzerland: amine fluoride (AmF, Olaflur/Dectaflur, 0.5% and 1% F), sodium fluoride (NaF, 0.5% and 1% F), each at pH 3.9 and 7.0, and stannous fluoride (SnF₂, 0.5% and 1% F), at pH: 3.9. The solutions were equimolar with respect to fluoride (5000 or 10000 ppm). The stannous fluoride solutions were prepared as acidic formulations only, as the neutral solutions were not stable with respect to SnF₂ and lead to formation of SnF₂(OH)₂ and SnO₂ within few hours (information of GABA International). The pH of the solutions was adjusted with hydrochloric acid or sodium hydroxide, respectively. Prior to the experiment, the pH and fluoride concentration of all solutions were checked. The pH of the acid solutions amounted to 3.7 to 4.0, while the pH of the neutral solutions amounted from 6.8 to 7.0. The fluoride concentration of the 0.5 % and 1 % fluoride solutions amounted to 0.49 to 0.51% and 0.96 to 1.11 %, respectively.

Two fluoride-free solutions (pH: 3.9 and 7.0) with the same basic formulation served as placebo.

**Pellicle formation and fluoride treatment**

Over three days, the enamel samples each group were submitted to a cyclic de- and remineralisation (6 cycles daily) in the so-called artificial mouth. Each day, the samples were exposed in vitro to pooled human saliva for 120 min (37°C) prior to the fluoride treatment and the insertion in the artificial mouth. For pellicle formation, each 12 specimens (which were applied afterwards in the artificial mouth) were randomly chosen and immersed in 5 ml freshly collected pooled saliva without agitation. Unstimulated human saliva was collected between 8 a.m. and 8.30 a.m. from 5 (2 male/ 3 female) healthy volunteers aged between 20 and 30 years. The volunteers had no active carious lesions, erosions or salivary dysfunction and did not use any kind of medication. Two hours before and during collection of the saliva the subjects were advised not to eat or drink. Ethic approval for the saliva collection was granted by the institutional ethics committee.
Pellicle-covered samples were treated with 50 µl of the respective fluoride or placebo solution for 3 min. After treatment, specimens were rinsed with distilled water for 15 s and stored in artificial saliva for 1 h prior to the insertion in the artificial mouth. Pellicle-covered enamel specimens which were not treated with any solution served as control.

**Cyclic de- and remineralisation**

De- and remineralisation in the artificial mouth included six daily erosive attacks (each 90 s) with hydrochloric acid (pH: 2.6) intermitted of each 1 h remineralisation in artificial saliva prepared accordingly to the formulation of Klimek et al.\textsuperscript{12} The artificial saliva was renewed daily. After the six daily cycles, the samples were stored in artificial saliva up to the next day. The artificial mouth consisted of 12 chambers in which the specimens were fixed. Each chamber was connected to two multichannel pumps (IPC/IPC-N Kassetten-Schlauchpumpen, Ismatec SA, Glattbrugg-Zürich, Switzerland), which allow for alternating rinsing of the samples with two different liquids. For rinsing the samples, the liquids were pumped from a reservoir outside of the chambers into a channel of 1 mm height which was located between the surface of the enamel specimens and the top of the chambers. Temperature and pumps were controlled by a computer and customized software. The specimens were randomly distributed to the chambers in each pH cycle.

Prior to the demineralisation, distilled water was rinsed through the channels (0.9 ml/min, 60s) to ensure a bubble-free flow of the solutions. Moreover, due to the small height of the space, continuous flow of the acid without generation of bubbles resulted. The chambers were aligned in an angle of 30° to the horizontal which allowed the acids to flow through the channel to the outlet of the chamber. The flow rates of hydrochloric acid and artificial saliva amounted to 3 ml/min and 0.5 ml/min, respectively.
Profilometry

Enamel loss was quantitatively determined using a profilometer (Perthometer S2, Mahr, Göttingen, Germany) with a diamond stylus moving across the eroded enamel surface and the reference areas (acrylic resin). Prior to the experiment, five baseline surface profiles were obtained from all specimens as references for calculating enamel loss. After the experiment, profilometric analysis was performed again, and the average depth of the eroded surface relative to the baseline surface profiles was calculated by the corresponding software (Mahr Perthometer Concept 7.0, Mahr, Göttingen, Germany). Since the enamel samples could be exactly repositioned in the wells of the profilometer, matching of the respective baseline and final profiles was possible.

Statistical analysis

Mean and standard deviation (SD) of enamel loss in each group was calculated. Kolmogorov-Smirnov test was applied to all groups to prove the normality assumption. Statistical analysis by three-way ANOVA considering the fluoride compound, fluoride concentration and pH of the solutions could not be conducted due to the unbalanced study design (SnF₂ solutions only available at acidic and not at neutral pH). Thus, two-way ANOVA was applied to the data, separately for the acidic and neutral fluoride solutions, respectively, considering both the fluoride compound and fluoride concentration as independent variables.

Finally, one-way ANOVA for comparison among all groups including the control and the placebos was conducted. One-way ANOVA was followed by Bonferroni-Dunn and Dunnett post hoc tests. The level of significance was set at p < 0.05.

Results

Mean enamel loss ([μm] ± SD) of all experimental groups is presented in Table 1. Kolmogorov-Smirnov test revealed that the assumption of normality in each group was not violated.
Two-way ANOVA of the acidic fluoride solutions found the fluoride compound but not the fluoride concentration to be significant with respect to enamel loss. As a significant interaction between the factors “fluoride compound” (3 levels) and “fluoride concentration” (2 levels) was found, one-way ANOVA for “fluoride compound x fluoride concentration” (factor with 6 levels) was computed. Bonferroni-Dunn post hoc tests revealed that 1% NaF was significant different from 1% AmF, 0.5% SnF$_2$ and 1% SnF$_2$, while the other groups were not significantly different from each other.

In contrast, two-way ANOVA of the neutral solutions found no factor (fluoride compound and fluoride concentration) to be significant with respect to enamel loss.

Enamel loss in the control group amounted to 2.3 µm ± 0.8. One-way ANOVA showed that only the acidic 0.5% and 1% SnF$_2$ solutions and the acidic 1% AmF solution reduced enamel loss significantly compared to the control, while all other groups were not significantly different from the control. Among group comparisons revealed no significant differences of the acidic 0.5% SnF$_2$, 1% SnF$_2$ and 1% AmF solutions.

**Discussion**

The present study was designed to analyze the protective efficacy of different fluoride compounds in acidic and neutral solutions on enamel erosion.

In order to simulate clinical conditions with the presence of an acquired pellicle on the tooth surface, the samples were pretreated with pooled human saliva each day to allow for the formation of an in vitro pellicle-like layer. However, it has to be taken into consideration that this pellicle-like layer formed in vitro might not reflect the bioadhesion occurring in vivo.

As shown by Rosin-Grget et al.\textsuperscript{16} and Larsen and Richards\textsuperscript{14}, saliva pretreatment of enamel samples might enhance the formation of KOH-soluble fluoride on enamel after application of AmF or NaF. However, even though the calcium content presented in the salivary pellicle\textsuperscript{7} might contribute to an increased formation of CaF$_2$, this fact might be of less relevance in the case of
acidified fluoride solutions, which lead to an increased release of calcium ions from enamel and thus, to higher calcium concentrations available for the formation of CaF$_2$ than in saliva.

Within the limitations of an in vitro study, the de- and remineralisation cycles in the artificial mouth intended to simulate clinical conditions with the short time consumption of acidic beverages several times daily. Even though it is known that an intensive fluoridation of dental hard tissues would be more effective in reducing erosion progression, the samples were submitted only one time daily to the fluoride or placebo solutions to simulate a patients single daily use of highly-fluoridated oral hygiene products.

As shown previously, the acidic SnF$_2$ solutions exhibited a distinct protective potential on enamel erosion. The efficacy of the SnF$_2$ solutions might only partly attributed to the CaF$_2$ layer, but also to the reaction of the tin ion with the hydroxylapatite lattice. In previous studies it was shown that Sn$_2$OHPO$_4$, Sn$_3$F$_3$PO$_4$, Ca(SnF$_3$)$_2$ and CaF$_2$ are possible reaction products of SnF$_2$ and hydroxylapatite. By SEM analysis it could be shown that SnF$_2$ or SnCl$_2$ treatment lead to a tin rich coating on enamel surfaces. This coating is still visible after 2-min immersion in citric acid (pH 2.3). Thus, it might be assumed that these Sn-containing reaction products play a decisive role for the erosion-inhibiting effect of the SnF$_2$ solutions in the present study. It might be speculated that the erosion-inhibiting effect of SnF$_2$ might be enhanced when the solution is applied frequently. It was shown that SnF$_2$ inhibited enamel erosion completely, when the solution was applied immediately after each erosive attack.

The efficacy of the acidic amine fluoride solution might be mainly related to the formation of the CaF$_2$-like layer, which is formed on enamel surfaces even within a short exposure time of 20 s to 2 min. As the formation of the CaF$_2$-layer is enhanced under acidic conditions, acidic fluoride solutions might be generally more effective than neutral solutions. Interestingly, the acidic NaF solutions failed to reduce erosive enamel loss significantly, although Schlueter et al. showed that an acidic NaF solution (1% F, pH: 1.2) was able to reduce mineral loss of eroded enamel. In contrast to the results of the present study, Ganss et al. demonstrated that AmF had no significant
effect on enamel erosion, while NaF decreased mineral loss significantly (both solutions: 250 ppm F⁻, pH: 3.5).

However, Petzold¹⁵ found no difference in the quantity, structure and composition of the precipitates formed on enamel surfaces after application of acidic NaF and AmF solutions. Own unpublished data showed that the amount of KOH-soluble fluoride on pellicle-covered enamel was slightly but not significant higher in samples treated with 1% AmF compared to 1% NaF²⁰. One possible explanation for the better efficacy of AmF compared to NaF might be the cationic nature of AmF, which might lead to a better adherence of the fluoride precipitates on the pellicle-covered surface.²⁰ The low pH of the solution might favour the ionization of AmF and, consequently, the interaction of this compound with proteins present on the pellicle-covered surface. De Jong et al.²,³ showed that AmF offered a better capability to adsorb on enamel and pellicle-covered enamel than NaF.

Generally, the neutral AmF and NaF solutions were not able to reduce enamel erosion significantly. Petzold¹⁵ showed that the application of NaF and AmF dissolved in distilled water on enamel for 2 min did not cause any visible precipitation. The earliest formation of scattered globules could be observed after about 60 min. Therefore, it is suggested that the application time used in the present study (3 min) was too short to allow for the formation of protective CaF₂-like layer, when the neutral solution was used. Moreover, the short application time might account for the fact that, generally, the protective potential of the 0.5% and 1% solutions was not significantly different. Although the 0.5% and 1% solutions differ in their amount of free fluoride ions, the solutions were applied at a comparatively high volume, which allows only a fraction of the free fluoride ions to react with the samples surface during the short application time.

In conclusion, at the same concentrations, acidic SnF₂ and AmF were shown to be more effective than NaF to protect enamel against erosion. Thus, the working hypothesis that the different fluoride solutions will not differ in their potential to reduce enamel erosion is rejected.
Acknowledgement

The authors thank Dr. Malgorzata Roos for statistical advice and GABA International for providing the test solutions.
References


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Tab. 1

Mean enamel loss ([µm] ± SD) of eroded samples treated with the different fluoride solutions. Only acidic 0.5% and 1% SnF$_2$ and 1% AmF solution led to a significant reduction of enamel loss compared to the control (marked by *).

<table>
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<th>Group</th>
<th>F concentration (%)</th>
<th>pH</th>
<th>Enamel loss ([µm] ± SD)</th>
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<tr>
<td>SnF$_2$</td>
<td>0.5</td>
<td>acidic</td>
<td>0.6 ± 1.1*</td>
</tr>
<tr>
<td>SnF$_2$</td>
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<td>acidic</td>
<td>0.6 ± 0.8</td>
</tr>
<tr>
<td>AmF</td>
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<td>acidic</td>
<td>1.1 ± 1.0</td>
</tr>
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<tr>
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<td>1.2 ± 1.1</td>
</tr>
<tr>
<td>NaF</td>
<td>1</td>
<td>acidic</td>
<td>2.3 ± 0.8</td>
</tr>
<tr>
<td>Placebo</td>
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<td>acidic</td>
<td>3.0 ± 1.4</td>
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<td>NaF</td>
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<td>1.4 ± 1.0</td>
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<td>2.0 ± 1.3</td>
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</tr>
<tr>
<td>Control</td>
<td>_</td>
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<td>2.3 ± 0.8</td>
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