Influence of fluoride concentration and ethanol pre-treatment on the reduction of the acid susceptibility of enamel

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Abstract

OBJECTIVE: To determine the association between KOH-soluble and structurally bound fluoride uptake and the erosion resistance of enamel, respectively. Additionally, the effect of enamel pre-treatment with ethanol before fluoridation was assessed. METHODS: Sixty bovine incisors (4 specimens/tooth) were randomly allocated to six groups (A-F). Samples 1 and 2 remained untreated, serving as control at baseline. Pre-treatment of the samples was performed for 5 min with 99% ethanol (groups A, B and C) or physiologic saline (groups D, E and F). Samples 3 and 4 were treated either with 0.5% (groups A and D), 1.0% (groups B and E) or 1.5% (groups C and F) fluoride solution. In samples 1 and 3, uptake of KOH-soluble and structurally bound fluoride was determined. Samples 2 and 4 were used for the determination of acid susceptibility by immersion in 1 ml HCl for 30s. Calcium release into HCl was assessed by atomic absorption spectroscopy. Differences between the groups were calculated by unpaired t-tests (p<0.05). RESULTS: Mode of pre-treatment showed no influence on fluoride acquisition. KOH-soluble and structurally fluoride uptake increased with increasing fluoride concentrations. Highest acid resistance was observed after treatment with 1% fluoride solution for both kinds of pre-treatment followed by 1.5% and 0.5% fluoride solution. CONCLUSION: Dose-dependency was observed for enamel fluoride acquisition but not for acid resistance.
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Running title: Calcium loss after fluoride application

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Abstract

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Results: Mode of pre-treatment showed no influence on fluoride acquisition. KOH-soluble and structurally fluoride uptake increased with increasing fluoride concentrations. Highest acid resistance was observed after treatment with 1% fluoride solution for both kinds of pre-treatment followed by 1.5% and 0.5% fluoride solution.

Conclusion: Dose-dependency was observed for enamel fluoride acquisition but not for acid resistance.
Introduction:

The prevalence of caries has declined over the last years in developed countries.\(^1\) In contrast to dental hard tissue loss due to caries, which is associated with the presence of microorganisms\(^2-4\), erosions are defined as dissolution of dental hard tissue by acids in absence of microorganisms.\(^5\) Over the last years, the loss of dental hard tissue due to erosion has increased.\(^6,7\) Beside the direct loss due to the dissolution of dental hard tissue, the previously eroded enamel or dentine is more susceptible to mechanical abrasion by toothbrushing and other mechanical impacts.\(^8-11\)

There are different approaches to prevent erosive tooth wear. On the one hand it might be possible to reduce the erosive potential of different erosive substrates. Spencer and Ellis demonstrated already in 1950 that the erosive potential of grapefruit juice is reduced when fluoride is admixed.\(^12\) Also the admixture of monocalcium phosphate and sodium phosphate reduces the erosive potential of beverages.\(^13\) Despite the positive effects of the admixture of fluoride, it must be taken in consideration, that it is not feasible to add an appropriate amount of fluoride to a beverage due to systemic toxicological reasons.\(^14\)

Another possible approach to decrease erosive tooth wear is to reduce the susceptibility of enamel due to acids by topical application of fluorides or use of fluoridated toothpastes. Davis and Winter\(^15\) showed in 1977 that the use of fluoridated toothpaste before an erosive attack reveals a protective effect of about 20\%. The application of topical fluoride agents on enamel results in the formation of a calcium fluoride-like precipitate.\(^16\) As suggested by Ganss et al.\(^17\), the calcium fluoride-like layer provides more minerals to the acid, to be dissolved before the underlying enamel is affected. Another conceivable approach, is that the calcium fluoride-like precipitate acts as a diffusion barrier hampering the contact of acids with
the dental hard tissues. A thicker layer of calcium fluoride-like precipitate may result in a better protection of the underlying enamel against erosion. It may be speculated whether a higher concentration of fluoride forms a thicker layer of calcium fluoride-like precipitate and so inhibits demineralization of the underlying enamel better. There are different studies showing that a higher concentration of fluoride does not necessarily result in a significant higher amount of KOH-soluble and structurally bound fluoride. These studies were performed concerning the caries preventive effects of the calcium fluoride-like precipitate. The protective effect against erosions was not evaluated.

Previous pilot studies in our laboratory revealed that the uptake of KOH-soluble fluoride is enhanced when the enamel was pre-treated with ethanol before the application of fluoride solution (unpublished data). The hypothesis was that the enamel will be dehydrated by the application of ethanol, analogue to the dehydration of histological samples with ethanol prior to the immersion in embedding materials, which results in an enhanced uptake of water containing fluoride solutions. It might be suggested that dentists could also perform this pre-treatment, thus enhancing the acid resistance of the so treated enamel.

Therefore, the aim of this study was to evaluate the influence of the concentration of amine fluoride and pre-treatment of enamel with ethanol on the uptake of KOH-soluble and structural bonded fluoride and on the resistance of enamel due to erosion.

The hypothesis was, that a higher concentration of fluoride results in a thicker layer of calcium fluoride-like precipitate and a higher amount of structural bonded fluoride and that the acid resistance of the enamel increases with increasing fluoride concentration of the topical application.
Material and methods:

Sample preparation

Sixty freshly extracted bovine lower incisors were randomly assigned to six groups (A-F). The teeth were sectioned at the cementum-enamel junction with a water-cooled diamond coated disc. The pulp tissue of the crown was removed with endodontic files. From the buccal surface of each crown four cylindrical samples (1-4) were prepared with a trephine drill with an inner diameter of 3 mm. These enamel cylinders were marked in order to identify respective samples of one tooth during the whole treatment process. The enamel cylinders were placed with the enamel surface downwards in cylindrical aluminium sample moulds with an inner diameter of 5 mm and embedded in acrylic resin (Palavit G, Kulzer Wehrheim, Germany). After removing the samples from the moulds, the enamel surface was ground with abrasive paper (800, 1000, 1200, 2400 and 4000 grit; Water Proof Silicon Carbide Paper, Struers, Erkrath, Germany). During these grinding steps the outermost 200 µm of enamel was removed. The loss of enamel was controlled with a micrometer (Mitutoyo, Tokyo, Japan).

Study design and allocation

For the pre-treatment, samples of groups A-C were stored in 1 ml of 99% ethanol for 5 min while the other samples were stored in physiologic saline for 5 min (D-F).

Samples “1” remained untreated and were used to determine the baseline content of KOH-soluble and structurally bound fluoride of the respective teeth. Samples “2” were used to measure the acid susceptibility of the untreated enamel. Samples “3 and 4” of groups A and D were treated with 0.5%, of groups B and E with 1.0% and of groups C and F with 1.5% amine (Olafur) fluoride solution (experimental solutions, GABA International AG, Therwil, Switzerland). The samples were covered with 1 ml
of the respective fluoride solution for 5 min. The pH of the fluoride solutions was adjusted to 3.9 by adding HCl and NaOH. After incubation, the samples were rinsed with tap water for 10 min and the uptake of KOH-soluble and structurally bound fluoride of samples “3” was determined. The samples “4” were used to measure the acid susceptibility of the enamel after fluoride treatment. In table 1 a short overview about the pre-treatment and treatment in the different groups is given. A specific overview of the treatment in the groups is exemplarily shown for group A in diagram 1.

Fluoride determination and acid resistance
The amount of KOH-soluble fluoride of samples 1 and 3 was determined with the method established by Caslavska et al.22 Each sample was stored in an Eppendorf Tube (Eppendorf International, Hamburg, Germany) with 1 ml 1mol/l KOH for 24 h under constant motion and the fluoride content in the solution was measured with a fluoride electrode.
After measurement of the KOH-soluble fluoride, the amount of structurally bound fluoride of the samples “1 and 3” was assessed by an acidic biopsy described in detail by Schmidlin et al.23
For determination of the acid susceptibility of the enamel, the samples 2 and 4 were immersed in 1 ml HCl for 30 s. During this time, the acid was gently stirred. The pH of the acids was adjusted to 2.6 by admixing distilled water. After removing the samples from the acid, 0.5 ml of the acid was mixed with 0.5 ml H2O and 0.5 ml strontium chloride (0.25%). The strontium chloride was used to mask the solved phosphate, which otherwise affects the following calcium determination by atomic absorption spectroscopy (2380 Atomic Absorption Spectrophotometer, Perkin-Elmer,
Schwerzenbach, Switzerland). The amount of calcium in this solution was measured at 422.7 nm.

*Data presentation and statistical analysis*

For calculating the uptake of KOH-soluble and structurally bound fluoride, the measured baseline contents (sample 1) were subtracted from the measured amounts after fluoride application (sample 3 of the respective tooth). If the measured baseline content was under the detection limit of 4 mmol/l fluoride of the fluoride electrode\(^{24}\), the amount was set as 0 mmol/l.

To determine the increase of acid resistance of enamel due to the application of fluoride, the amount of calcium in the acid of sample “2” (baseline) was divided by the amount of calcium of the respective sample “4” (treated with fluoride). This factor will be later on referred to as resistance factor. A factor > 1 means that the acid resistance of the treated samples was higher than the acid resistance of the respective baseline control. Moreover, reduction of the acid susceptibility was associated with an increase of the factor.

Mean values and standard deviation was calculated for data presentation.

Data analysis was performed using ANOVA followed by Bonferroni/Dunn post-hoc tests. For calculating differences between the groups concerning the amount of KOH-soluble and structurally bound fluoride and the reduction of the acid susceptibility, unpaired t-tests were used. Significance level was set at 95%.

**Results:**

*Uptake of KOH-soluble fluoride*

The uptake of KOH-soluble fluoride is shown in figure 1.
The uptake of KOH-soluble fluoride between the two different kinds of pre-treatment was not statistically significant different when the samples were later treated with fluoride solution of the same concentration. For both kinds of pre-treatment an increase in the amount of KOH-soluble fluoride with a rising concentration of fluoride solution was noticeable. Comparing the uptake of KOH-soluble fluoride only for the samples, which were pre-treated with ethanol, significant differences between the samples treated with 0.5 % and 1.0 % (p=0.0377), 0.5% and 1.5% (p=0.0003) and 1.0% and 1.5% fluoride solution (p=0.0042) were found. When comparing the samples which were pre-treated with physiologic saline, only the samples later treated with 0.5% and 1.5% fluoride solution showed statistically significant differences in the amount of KOH-soluble fluoride (p=0.0342).

**Uptake of structurally bound fluoride**

As shown in Fig. 2, there was no statistically significant difference in the uptake of structurally bound fluoride between the two different kinds of pre-treatment when the samples were later treated with fluoride solution of the same concentration. The amount of structurally bound fluoride increased with the rising concentration of fluoride solution for both kinds of pre-treatment. The uptake of structurally bound fluoride of the samples pre-treated with ethanol and later treated with 0.5% and 1.0% of fluoride solution was not statistically significantly different (p=0.6954). However, statistically significant differences were found between the samples later treated with 0.5% and 1.5% (p=0.0206) and with 1.0% and 1.5% fluoride solution (p=0.0422), respectively.

There were no statistically significant differences between the uptake of structurally bound fluoride for the samples pre-treated with physiologic saline and later treated with 0.5% and 1.0% (p=0.1140) and with 1.0% and 1.5% of the fluoride solution
(p=0.0550), while the 0.5% and 1.5% fluoride solution were significant different (p=0.0020).

Resistance factor
The acid resistance factors (Fig. 3) were not statistically significantly different for the two kinds of pre-treatment, when the samples were later treated with the same concentration of fluoride solution.

The resistance factors of the samples pre-treated with ethanol and either treated with 1.0% or 1.5% fluoride solutions were not statistically significantly different (p=0.8647). In contrast, the differences of the resistance factor of the samples treated with 0.5% fluoride solution compared with the acid resistance factors of the samples treated with 1.0% or 1.5% fluoride solution were statistically significant (p=0.0204 and p=0.0124), respectively.

For the samples pre-treated with physiologic saline no statistically significant difference in the resistance factors of the samples treated with either 0.5% or 1.5% was recorded (p=0.0737). Also the acid resistance factors of the samples treated with 1.0% and 1.5% fluoride solution were not statistically significantly different (p=0.1064). Only the resistance factors of the samples treated with 0.5% and 1.0% fluoride solution were statistically significantly different (p=0.0038).

Discussion
Enamel samples of the present study were gained from bovine lower incisors because it is easier to obtain a sufficient number of bovine teeth and the size of the bovine lower incisors makes it possible to gain more then one sample from one tooth, thus having untreated control samples for each tooth. Studies comparing the mineral content and mineral distribution in human and bovine enamel have found no
significant difference for these two different substrates. Also other studies have used bovine enamel to substitute human enamel when investigating interaction of fluorides with enamel and the formation of calcium fluoride-like precipitate. The uptake of KOH-soluble fluoride was measured following the method described by Caslavska et al. Fluoride uptake has been detected with this method in numerous other studies. As the method by Caslavska et al. is well established in our laboratory and the sensitivity of the Raman spectroscopy is not high enough to measure the uptake of calcium fluoride-like precipitate after the application of certain kinds of fluoride, the method by Caslavska et al. was used in this study. The uptake of structurally bound fluoride was detected by analysis of the fluoride content of hydrochloric acid previously applied on the respective enamel samples. This successive acid etch biopsy has been used in different other studies. The samples of groups A-C were pre-treated with ethanol to enhance the uptake of fluoride by enamel. The enamel pre-treated with ethanol showed slightly higher uptake of KOH-soluble fluoride, even if these findings were not statistically significant. This finding is in accordance with the hypothesis that the uptake is enhanced by pre-treatment with ethanol. The reason for the observation that the higher uptake was not statistically significant might be referred to the low amount of water in enamel causing a minor effect of dehydration by ethanol. The results of this study show that the uptake of KOH-soluble fluoride was depending on the concentration of the applied fluoride solutions independent the kind of pre-treatment. This finding is in agreement with the finding by Rosin-Grget at al., even their findings were not statistically significant. To form calcium fluoride-like precipitate, the availability of calcium ions, independent of their origin (e.g. enamel, saliva or dental plaque) is an important factor. As the pH of the present fluoride solutions was adjusted to 3.9 it can be assumed, that calcium ions are released from
the enamel the same way it occurs during erosion. These calcium ions are ready for reaction with the fluoride applied with the fluoride solutions. As long as enough calcium ions are released from the enamel due to the low pH of the solutions, higher fluoride concentrations of the applied solutions consequently form more calcium fluoride. In contrast to the findings by Rosin-Grget et al., this evaluation revealed significant differences in the amount of formed KOH-soluble fluoride, increasing with the concentrations of the applied fluoride solutions (0.5, 1.0 and 1.5%). This can be explained by the pH-value of the solutions. The pH-value of the solutions used in the present study was lower than the pH-values of the solutions used by Rosin-Grget et al. and so more calcium ions will be released from the enamel. Thus, more fluoride ions were able to form calcium fluoride like precipitates, resulting in an increase of calcium fluoride-like precipitate formation with increasing fluoride concentrations.

For the structurally bound fluoride a similar finding could be observed. In the present study, the uptake of structurally bound fluoride showed a dose dependency with the concentration of the applied fluoride solutions, what is comparable with the findings of other studies also there results were again not statistically significant. These minor differences could be again explained with the difference in the pH-values used in these studies.

The resistance factors measured were in the range of the reductions of erosive tooth wear due to the application of fluoride found in previously studies. With regard to the resistance factor, the present study shows unexpected results. The resistance factor does not increase, when the highest concentration of fluoride (1.5%) was applied as compared to the application of 1% fluoride solution. This finding could be explained with the chemical composition of the KOH-soluble fluoride. This calcium fluoride-like precipitate, which is KOH-soluble, is formed from the fluoride solution applied and from calcium released from the enamel during the application of the
In this study, the amount of KOH-soluble fluoride was highest, when fluoride solutions with a concentration of 1.5% were applied. Thus a high amount of calcium is existent on the surface of these samples. During an acidic challenge, this calcium is released to the acid as the dissolution rate of calcium fluoride-like material is higher than the dissolution rate of enamel. This means, that the samples with the higher amount of calcium fluoride-like precipitate show a higher release of calcium compared with their untreated control samples than the samples treated with lower fluoride concentration. One might argue that this calcium is from the calcium fluoride-like precipitate and not from the underlying enamel, but it has to be taken in consideration that this calcium must be released from the enamel previously during the application of fluoride solutions as the solutions does not contain calcium and no other source of calcium like saliva was present in this study. Thus, the loss of calcium happens in these samples already during the application of fluoride and with the dissolution of the calcium fluoride-like precipitate during erosion, the calcium was finally lost from the enamel. It might be speculated, that the situation in situ might be different as the saliva also provides calcium to form the calcium fluoride-like precipitate, but there are other studies showing, that even under the presence of saliva, no additional calcium fluoride is formed. To elucidate these differences, further in situ studies should be performed.

The results of this study showed dose-dependency between concentration of the applied fluoride and the formed amount of KOH-soluble and structurally bound fluoride. However, no such dose-dependency could be found between the concentration of the applied fluoride and the acid resistance of the so treated enamel. Measuring the calcium release into HCl tested the resistance of the treated enamel towards acidic dissolution. It is important to notice that the calcium measured in the solution might be originated from both the calcium fluoride-like layer and the
underlying enamel. However, since the calcium in the calcium fluoride-like layer also originated from the enamel, the resistance factor mirrors the susceptibility of the enamel to release calcium after fluoride treatment.

Conclusion

In general in might be concluded that 1) ethanol pre-treatment does not effect enamel fluoride uptake an 2) the uptake of KOH-soluble and structurally bound fluoride shows dose-dependency while for the improvement of acid resistance no such dose-dependency exist.

Acknowledgment

The authors thanks GABA International AG for providing us with the fluoride solutions.
References:


Figure Captions

Tab.1: Pre-treatment and treatment in the groups A-F.

Dia. 1: Treatment of the samples within the groups exemplarily shown for group A.

Fig. 1: Uptake of KOH-soluble fluoride after pre-treatment with either ethanol or physiologic saline and treatment with 0.5, 1.0 and 1.5% amine fluoride solution. Groups with the same kind of pre-treatment, which were not statistically significantly different, are marked with same letters.

Fig. 2: Uptake of structurally bound fluoride after pre-treatment with either ethanol or physiologic saline and treatment with 0.5, 1.0 and 1.5% amine fluoride, respectively. Groups with the same kind of pre-treatment, which were not statistically significantly different, are marked with same letters.

Fig. 3: Resistance factor of samples treated with 0.5, 1.0 and 1.5% amine fluoride solution after pre-treatment with either ethanol or physiologic saline. A factor > 1 means that the acid susceptibility of the treated samples was lower than the susceptibility of the respective untreated sample. The higher the factor, the higher the acid resistance was. Groups with the same kind of pre-treatment, which were not statistically significantly different, are marked with same letters.
Fig. 1:
Fig. 2:
Fig. 3:
<table>
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<tr>
<th>Group</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
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<td>1.0% fluoride solution</td>
<td>1.5% fluoride solution</td>
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Tab. 1:
Group A
(n = 10 teeth)

Preparation of 4 samples (1-4) per tooth

Samples “1”
n = 10
Samples “2”
n = 10
Samples “3”
n = 10
Samples “4”
n = 10

Pre-treatment of each sample in 1 ml 99% ethanol for 5 min
(pre-treatment as stated in Tab. 1)

Treatment of each sample in physiologic saline for 5 min
Treatment of each sample with 1 ml 0.5% fluoride solution for 5 min
(treatment as stated in Tab. 1)

Rinsing of the samples for 10 min with tap water

Determination of KOH-soluble and structurally bound fluoride
Determination of acid susceptibility
Determination of KOH-soluble and structurally bound fluoride
Determination of acid susceptibility

Dia. 1: