

# Effects of Two Fluoridation Measures on Erosion Progression in Human Enamel and Dentine in situ

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## Key Words

Dentine · Enamel · Erosion · Fluoride

## Abstract

The aim of the present study was to evaluate the effects of fluoride on erosive mineral loss in human enamel and dentine using a cyclic de- and remineralisation model in situ. The study was a three-treatment (5 days each) crossover design involving 4 (enamel) or 6 (dentine) healthy volunteers. Samples were recessed in palatal mouth appliances and worn day and night except during meals and were demineralised extraorally with 0.05 M citric acid (pH 2.3) for 6 × 5 min daily. Fluoridation was performed with toothpaste (SnF<sub>2</sub>/Olaflur; 0.14% F<sup>-</sup>) for 3 × 5 min daily (toothpaste fluoridation) or with toothpaste in combination with a mouthrinse (SnF<sub>2</sub>/Olaflur; 0.025% F<sup>-</sup>) for 3 × 5 min daily and with a gel (NaF/Olaflur, 1.25% F<sup>-</sup>) on days 1 and 3 instead of the toothpaste (intensive fluoridation). In the control group no fluoridation was performed. Mineral loss (µm) was determined with the use of longitudinal microradiography. In enamel, mineral loss was 40.7 ± 15.1 µm in the control group, 18.3 ± 12.4 µm after toothpaste fluoridation and 5.0 ± 12.2 µm after intensive fluoridation. The respective

values for dentine were 49.0 ± 15.4, 35.0 ± 15.5 and 19.8 ± 12.0 µm. All differences were statistically significant ( $p \leq 0.001$ ). The results indicate that intensive fluoridation is effective in preventing enamel and dentine from mineral loss even under severely erosive conditions.

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In vitro studies have demonstrated limited effectiveness of fluoride in protecting enamel from erosive mineral loss [Davis and Winter, 1977; Ganss et al., 2001]. In the case of dental erosion, no subsurface lesion is available for remineralisation, and therefore the precipitation of CaF<sub>2</sub>-like layers can be assumed to be the only mode of action. The formation and dissolution of CaF<sub>2</sub> and CaF<sub>2</sub>-like material are not straightforward processes and depend on several factors such as pH and the presence of phosphate [Christoffersen et al., 1988]. Under neutral conditions CaF<sub>2</sub>-like layers appear to be relatively stable, but dissolution is increased at lower pH [Lagerlöf et al., 1988]. At least in vitro, the protective effect of fluoride applications is therefore related to the thickness of the precipitated calcium fluoride salts. In saliva, however, CaF<sub>2</sub> is less soluble than in water or in phosphate buffer [Saxegaard et al., 1988] and CaF<sub>2</sub>-like precipitates can therefore be ob-

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served for several weeks after application, if a sufficient amount is deposited initially [Dijkman et al., 1983]. We therefore hypothesise that the efficacy of fluoride may be much better in vivo than in vitro.

In dentine, erosion progression is almost completely inhibited by the presence of high amounts of fluoride in vitro [Ganss et al., 2001]. During demineralisation, the organic dentine matrix is exposed [Kinney et al., 1995]. It can be speculated that this collagen-rich surface acts as a buffering membrane preventing deeper dentine layers from reaching low pH values and that this layer is essential for the fluoride effect. It is not known if the organic layer develops under in situ conditions where mechanical or enzymatic destruction of collagen is possible. It is therefore questionable if the promising results from the in vitro study can be reproduced under in situ conditions.

The aim of the present study was to evaluate if fluoride is capable of reducing the erosive demineralisation in enamel and dentine under in situ conditions.

## Materials and Methods

### *Sample Preparation and Mouth Appliances*

Freshly extracted human third molars that had not been exposed to the oral environment were used. The teeth were stored in saturated aqueous thymol solution until use. All donors lived in an area with  $\leq 0.3$  ppm fluoride in the drinking water. From each tooth three longitudinal enamel or dentine samples were prepared. From the enamel specimens, an outer surface layer of 300  $\mu\text{m}$  was removed and the samples were ground flat to a final thickness of 400  $\mu\text{m}$  using P 800 silicon carbide abrasive paper (Leco, St. Joseph, USA). Dentine samples were carefully checked for enamel remnants and ground to a thickness of 700  $\mu\text{m}$ . All cutting and grinding procedures were performed under sufficient water flow (Exact Trennschleifsystem and Exact Mikroschleifsystem, Exakt-Apparatebau, Norderstedt, Germany). The samples were embedded in a light-curing resin material (Technovit 7230 VLC, Kulzer-Exakt, Wehrheim, Germany) and mounted into sample holders for microradiographic procedures. A total of 8 embedded samples were recessed in cold-cured acrylic palatal mouth appliances retained by braces at the first upper molars. The sample surfaces were coplanar with the surface of the appliance.

For disinfection, the samples were stored in saturated aqueous thymol solution [Ingram et al., 1997; Amaechi et al., 1998] for at least 2 weeks. Before insertion, the appliances with the samples were immersed in 70% ethanol for 30 min. After this procedure, a microbiological evaluation revealed no cultivable microorganisms.

### *Subjects and Study Design*

The study was performed according to the guidelines of Good Clinical Practice and was approved by the local Ethical Committee (Ethik-Kommission des Fachbereichs Medizin der Justus-Liebig-Universität Giessen). All participants gave their informed consent. Inclusion criteria were good general and oral health, good oral hygiene (no visible plaque) as well as normal saliva flow rates and buffer capacity (range for all participants: resting saliva 0.23–

0.60 ml; stimulated saliva 1.4–2.2 ml; normal final pH in resting saliva 4.28–4.53 and in stimulated saliva 5.52–5.96).

The study was performed involving 4 (enamel) and 6 (dentine) volunteers using a crossover design with 3 treatment periods of 5 days each. The participants were extensively trained with all procedures; they also received a written protocol and a schedule. The appliances were worn during day and night (24 h) except for meals. After meals or drinks, 15 min elapsed before reinsertion. For erosive demineralisation, the mouth appliances were immersed extraorally in 40 ml 0.05 M citric acid (pH 2.3) for 6  $\times$  5 min daily and thoroughly rinsed with tap water before reinsertion. The interventions were evenly distributed over the day and performed after breakfast, in the morning, after lunch, in the afternoon, after dinner and before bed time, simulating three acidic meals and three acidic drinks or snacks daily. The demineralisation solution was renewed after each treatment period and pH monitored. After demineralisation, the samples were reinserted (controls) and in the experimental groups fluoridation was performed intraorally with 10 ml of mouthrinse (Olaflur/SnF<sub>2</sub>; 0.025% F<sup>-</sup>; Meridol®, Gaba AG, Therwil, Switzerland), or with toothpaste (Olaflur/SnF<sub>2</sub>; 0.14% F<sup>-</sup>; Meridol) or with gel (Olaflur/NaF; 1.25% F<sup>-</sup>; Elmex® gelee, Gaba AG) during standardised brushing. The volunteers first brushed the lower jaw for 30 s to produce a sufficient amount of saliva/toothpaste or saliva/gel slurry, which was then held in the oral cavity for 5 s for standardised spreading of fluoride over the palatal samples. Brushing was continued for a total brushing time of 5 min. The samples were not brushed. The appliances were rinsed under running water until free from visible remnants of toothpaste or gel and were immersed in the evening in a 0.1% chlorhexidine mouthrinse for 5 min to avoid plaque growth. Additional oral hygiene, if required, was performed with fluoride-free preparations. Treatment groups were defined as follows:

Group 1 (control): no fluoridation.

Group 2 (toothpaste fluoridation): fluoride toothpaste for 3  $\times$  5 min daily after the first, third and fifth demineralisation.

Group 3 (intensive fluoridation): fluoride toothpaste for 3  $\times$  5 min daily after the first, third and fifth demineralisation; fluoride solution for 3  $\times$  5 min daily after the second, fourth and last demineralisation; on days 1 and 3 gel fluoridation for 1  $\times$  5 min instead of the last toothpaste fluoridation.

Before and between the treatment periods, a 1-week period with fluoride-free oral hygiene procedures was included for washout; the order of treatment periods was different for all volunteers.

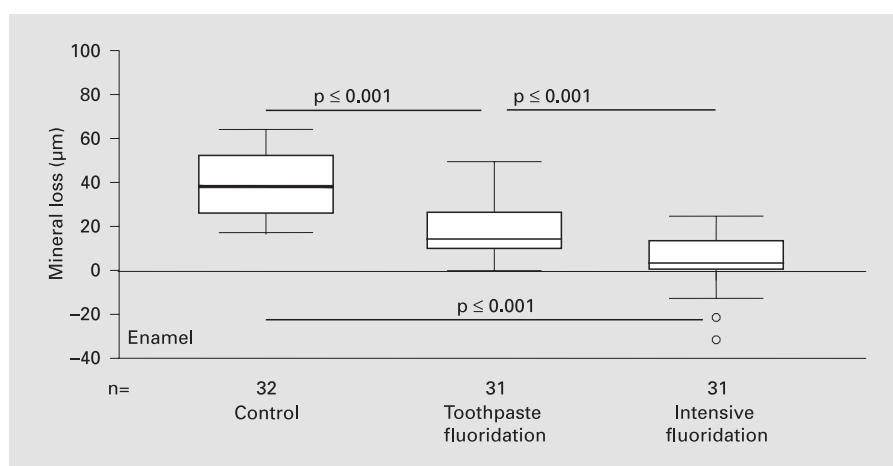
### *Microradiographic Procedures*

Mineral content was determined using longitudinal microradiography [de Josselin de Jong et al., 1988]. X-ray projection (CuK $\alpha$  X-ray radiation operated at 20 kV, 50 mA, exposure time 2.5 min) was made on a high-resolution film (high-speed holographic film, Kodak SO-253; Kodak, Stuttgart, Germany) and mineral content was densitometrically calculated at baseline. After the treatment period the embedded samples were removed from the appliances and repositioned in the respective sample holder for determination of the final mineral content. The values were calculated under the assumption that mineral content is 87 vol% for enamel and 47 vol% for dentine [Nikiforuk, 1985] and expressed in micrometres as the difference between baseline and final mineral content.

### *Statistics*

Statistical procedures were performed with the Statistical Package for Social Sciences (SPSS 10.0) for Windows 98. The data were

**Fig. 1.** Mineral loss ( $\mu\text{m}$ ) in enamel after 5 days of extraoral demineralisation and in situ fluoridation with toothpaste (toothpaste fluoridation), and with toothpaste, mouthrinse and gel (intensive fluoridation). Lines indicate significant differences between the groups. The boxes represent the 25.0 and 75.0 percentile with the inner line defined as median. Minimum and maximum values are represented by horizontal lines. o = Outliers.



**Table 1.** Mineral loss ( $\mu\text{m}$ , mean  $\pm$  SD) for each volunteer after 5 days of extraoral demineralisation without the use of fluoride (control), with in situ fluoridation with toothpaste (toothpaste fluoridation), and with toothpaste, mouthrinse and gel (intensive fluoridation)

	Volunteer 1	Volunteer 2	Volunteer 3	Volunteer 4	Volunteer 5	Volunteer 6
<i>Enamel</i>						
Control	46.6 $\pm$ 10.2	53.0 $\pm$ 11.2	27.5 $\pm$ 15.8	35.7 $\pm$ 9.4		
Toothpaste fluoridation	12.9 $\pm$ 9.7 <sup>b</sup>	33.4 $\pm$ 8.6 <sup>b</sup>	12.1 $\pm$ 7.0 <sup>a</sup>	14.2 $\pm$ 10.1 <sup>b</sup>		
Intensive fluoridation	8.7 $\pm$ 9.0 <sup>c</sup>	12.1 $\pm$ 10.9 <sup>c</sup>	3.4 $\pm$ 8.5 <sup>a</sup>	-3.6 $\pm$ 14.8 <sup>c</sup>		
<i>Dentine</i>						
Control	58.1 $\pm$ 15.1	38.9 $\pm$ 5.7	46.2 $\pm$ 14.2	47.9 $\pm$ 11.4	64.5 $\pm$ 13.5	38.7 $\pm$ 14.0
Toothpaste fluoridation	19.9 $\pm$ 6.4 <sup>c</sup>	31.8 $\pm$ 10.2 <sup>n.s.</sup>	29.7 $\pm$ 4.0 <sup>a</sup>	34.1 $\pm$ 17.1 <sup>n.s.</sup>	48.6 $\pm$ 12.8 <sup>a</sup>	48.6 $\pm$ 12.7 <sup>n.s.</sup>
Intensive fluoridation	22.1 $\pm$ 9.5 <sup>c</sup>	11.5 $\pm$ 8.8 <sup>c</sup>	19.3 $\pm$ 9.1 <sup>c</sup>	24.0 $\pm$ 20.0 <sup>a</sup>	22.3 $\pm$ 9.8 <sup>c</sup>	19.5 $\pm$ 10.4 <sup>b</sup>

The results of toothpaste fluoridation and intensive fluoridation were compared with controls; n.s. = not significant; <sup>a</sup>  $p \leq 0.05$ ; <sup>b</sup>  $p \leq 0.01$ ; <sup>c</sup>  $p \leq 0.001$ .

tested for normal distribution (Kolmogoroff-Smirnoff test). Since values were normally distributed in all groups, the t test for paired samples was used to determine differences between treatment groups on the individual level as well as for overall comparison. One-way analysis (ANOVA) was applied to describe interindividual differences. The level of significance was set at 0.05.

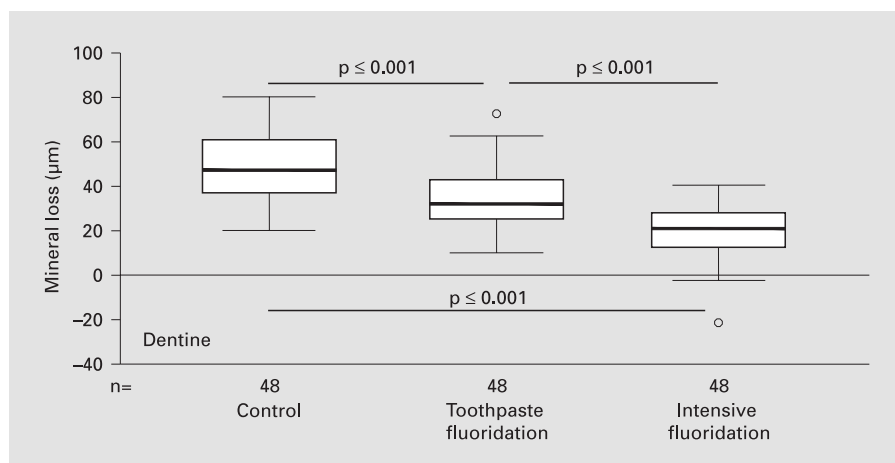
## Results

All of the volunteers completed the study satisfactorily. In some cases irritations of the oral mucosa were observed during intensive fluoridation, but recovered soon after the treatment period. Two samples were lost from the appliances; a total of 94 enamel and 144 dentine samples were analysed.

For enamel, the mean mineral loss in the control group was  $40.7 \pm 15.1 \mu\text{m}$ , which was significantly reduced after toothpaste fluoridation ( $18.3 \pm 12.4 \mu\text{m}$ ). After intensive fluoridation erosive mineral loss amounted to  $5.0 \pm 12.2 \mu\text{m}$ ; in some samples a mineral gain was observed (fig. 1). Between volunteers, significant differences were observed in the control group ( $p \leq 0.001$ ) as well as after toothpaste fluoridation ( $p \leq 0.001$ ), but not after intensive fluoridation (n.s.). In all volunteers, toothpaste fluoridation as well as intensive fluoridation significantly reduced mineral loss compared to the control group (table 1).

For dentine, mineral loss was  $49.0 \pm 15.4 \mu\text{m}$  in the control group,  $35.0 \pm 15.5 \mu\text{m}$  after toothpaste fluoridation and  $19.8 \pm 12.0 \mu\text{m}$  after intensive fluoridation

**Fig. 2.** Mineral loss ( $\mu\text{m}$ ) in dentine after 5 days of extraoral demineralisation and in situ fluoridation with toothpaste (toothpaste fluoridation), and with toothpaste, mouthrinse and gel (intensive fluoridation). Lines indicate significant differences between the groups. The boxes represent the 25.0 and 75.0 percentile with the inner line defined as median. Minimum and maximum values are represented by horizontal lines. o = Outliers.



(fig. 2). Similar to enamel, significant differences were found between volunteers in the control group ( $p \leq 0.001$ ) and the toothpaste fluoridation group ( $p \leq 0.001$ ), whereas intensive fluoridation reduced interindividual differences to a non-significant level. Compared to the control group, the application of toothpaste revealed a significant reduction of mineral loss in 3 of 6 volunteers, whereas intensive fluoridation was effective in all participants (table 1).

## Discussion

The present in situ study was designed following a previous in vitro study [Ganss et al., 2001] with a similar erosion and fluoridation protocol. Samples were demineralised extraorally to avoid mineral loss from the volunteers' teeth, whereas fluoridation was performed intraorally to simulate the clinical situation as closely as possible. To accumulate high amounts of  $\text{CaF}_2$ -like material, acidic fluoride preparations with prolonged application times, including a highly concentrated fluoride gel, were used. Fluoride was applied without brushing because a standardised brushing procedure especially with respect to brushing force is difficult to achieve in situ. The present fluoridation measure meets the clinical situation insofar as fluoride is delivered as a mouthrinse or as a gel using trays. Toothbrushing may abrade  $\text{CaF}_2$ -like material on enamel as was demonstrated in vitro, but  $\text{CaF}_2$ -like material is detected even after 150 brushing strokes with a relatively high brushing force [Attin et al., 2001]. This corresponds to the finding that high amounts of fluoride are also capable of reducing the amount of toothbrush abrasion of eroded tooth surfaces in situ [Ganss et al., 1999].

However, aspects of abrasion of eroded enamel and especially of eroded dentine are complicated and the clinical relevance of abrasion is still unclear. Considering the limited knowledge about the efficacy of fluoride in the context of dental erosion, the experiments were performed without additional brushing procedures.

The present in situ study has clearly shown that the topical application of fluoride significantly reduced erosive mineral loss in enamel even under severely erosive conditions. This is in contrast to Larsen and Richards [2002], who concluded from the good solubility of  $\text{CaF}_2$  in erosive drinks in vitro that a topical fluoride treatment given in vivo could not have a notable effect on erosion progression. Whilst the in vitro study mentioned above revealed a reduction of mineral loss of 10% after toothpaste fluoridation and 20% after intensive fluoridation [Ganss et al., 2001], the respective values were 50 and 90% in the in situ situation. In a number of samples (25%) a mineral gain was observed after intensive fluoridation even under severely erosive conditions.  $\text{CaF}_2$ -like material can be stabilised in the presence of relatively low concentrations of phosphate [Christoffersen et al., 1988] due to the adsorption of  $\text{HPO}_4^{2-}$  on the mineral surface [Lagerlöf et al., 1988]. The concentration of phosphate needed for stabilisation is substantially lower than usually found in oral fluids and the phosphate concentration in the remineralisation solution used in the in vitro study mentioned above was also similar to that in saliva. Therefore the role of salivary proteins has to be considered. In an in vitro experiment on the dissolution behaviour of calcium fluoride in different solutions, the dissolution of calcium fluoride during a 24-hour period was estimated to be 0.39 mg in a 2 mM phosphate buffer (pH 6.8) compared

to 0.26 mg in saliva supernatant, which was only a minor additional effect [Saxegaard et al., 1988]. It appears therefore questionable whether the adsorption of proteins either specifically or as a pellicle coating can explain the striking difference between in vitro and in situ results. Erosive demineralisation as well as its 'repair' by the precipitation of mineral in the presence of fluoride is a surface-controlled process and, under the complicated conditions in the oral environment, is not yet fully understood. Especially salivary factors, which have mostly been highlighted in the context of caries, have to be reconsidered regarding the nature of dental erosions.

In dentine, the results from the in vitro study were confirmed. In vitro, erosive mineral loss was reduced by about 10% after toothpaste fluoridation and by 55% after intensive fluoridation, which was effective enough to inhibit erosion progression completely after an initial period of demineralisation [Ganss et al., 2001]. This is probably related to the development of a demineralised collagen-rich surface layer acting as a buffering membrane, which protects deeper dentine layers from low pH values. Further erosive demineralisation and precipitation phenomena then become diffusion-controlled. In the presence of high amounts of fluoride, the buffering capacity of the organic material appears to be sufficient to decrease or inhibit further demineralisation. In the present in situ study, toothpaste fluoridation reduced mineral loss by 30% and intensive fluoridation by 60%. What was not demonstrated here is the erosion progression over the 5 days of treatment.

Unlike in vitro, however, the exposed dentin matrix is subject to chemical and mechanical processes under in situ conditions. The organic dentine matrix consists mostly of collagen [Linde, 1989], which can be solubilised non-specifically [Oyarzun et al., 2002] or by collagenase [Klont and ten Cate, 1991]. In the oral cavity, it is degraded by

bacterial enzymes when covered with plaque [van Strijp et al., 1992], but collagenase is also present in saliva [Gangbar et al., 1990; Uitto et al., 1990]. The clinical significance of intraoral collagenase in the context of dentin erosion, however, needs to be further clarified. It must also be pointed out that mechanical wear, as occurs with tooth brushing, may play a role. If the organic layer is more or less lost in the oral cavity, erosion and precipitation phenomena in dentine will tend to be surface-controlled with fluoride possibly merely acting as discussed in conjunction with enamel.

In both enamel and dentine experiments, significant differences between volunteers were observed in the control group and after toothpaste fluoridation but not after intensive fluoridation. It could be speculated that individual risk factors like the composition of saliva or fluoride clearance may play a role whereas in the presence of high amounts of fluoride these factors are compensated for by the high stability of these precipitates.

In conclusion, this study has demonstrated that high amounts of fluoride are capable of reducing erosive mineral loss in enamel and dentine effectively even under severely erosive conditions. Considering the striking difference of in vitro and in situ results, further studies should be performed with an in situ protocol. Further research is needed to elucidate the mode of action of fluoride and should also focus on the efficacy of different fluoride preparations and on dose finding. Clinical studies with relevant application modes are necessary to confirm the promising results from the in situ experiments.

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