

Formation of fluoride on enamel in vitro after exposure to fluoridated mouthrinses

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The aim of this study was to quantify the formation of alkali-soluble fluoride (loosely bound fluoride such as calcium fluoride-like material and absorbed fluoride) and alkali-insoluble fluoride (firmly bound fluoride or apatitically bound fluoride) when fluoride mouthrinsing solutions were applied on sound human enamel in vitro. Two commercial products containing 0.2% or 0.05% NaF were used during 30 sec, 60 sec, 5 min, and 60 min. The formation of loosely bound fluoride was determined by KOH extraction and visualized by scanning electron microscopy. The firmly bound fluoride was measured by three consecutive acid etchings of the enamel. Even during short periods of application there were deposits on the enamel surface. The amount of deposit increased with time of exposure to the 0.2% NaF solution. Only after treatment for 60 min with 0.05% NaF were significant amounts of alkali-soluble fluoride deposited. No measurable amounts of firmly bound fluoride were observed. □ *Alkali-insoluble fluoride; alkali-soluble fluoride; preventive dentistry; sodium fluoride*

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Fluoride-containing mouthrinses have become an accepted method for caries prevention in schoolchildren owing to the simplicity of use and the low cost involvement (1, 2). Many clinical trials using different fluoridated mouthrinse solutions have been performed, and caries reduction has also been obtained in subjects living in areas with non-fluoridated water supplies (3-7). Usually, neutral sodium fluoride solutions (0.2% or 0.05%) have been used, the higher concentration being designed for weekly and fortnightly use and the lower for daily use.

The mechanism of action of fluoride-containing mouthrinses is not well understood. It was assumed in the past that the fluoride reacted with the enamel and provided a more resistant surface. However, direct formation of fluorapatite is a slow process, and a more recent concept is that topical fluoride application causes deposition of a calcium fluoride-like material, which is subsequently incorporated into the enamel through demineralization/remineralization reac-

tions. The calcium fluoride-like material formed on teeth in vivo after topical application of fluoride consists mainly of calcium fluoride but also contains small amounts of phosphate, which renders it more soluble than chemically pure calcium fluoride. The calcium fluoride-like material is thought to be a pH-controlled reservoir of fluoride which releases fluoride mainly when the pH in plaque is low (8, 9).

Calcium fluoride or calcium fluoride-like material is soluble in alkali (1 M KOH for 24 h), whereas the firmly bound fluoride is alkali-insoluble (10). The aim of the present study was to quantify the formation of alkali-soluble fluoride, which comprises loosely bound fluoride or calcium fluoride-like material and adsorbed fluoride, and alkali-insoluble fluoride, which is firmly bound or apatitically bound fluoride, after a fluoride-containing mouthrinse was applied to human enamel surfaces in vitro. The treatment was carried out for different periods of time with 0.2% and 0.05% NaF solutions.

Materials and methods

Twelve surgically removed third molars were used in this experiment. They were cleaned with a rotating rubber cup and a pumice containing no fluoride, sprayed with distilled water, and inspected in a stereomicroscope. Specimens with surface defects were discarded.

The teeth were randomly divided into two groups of six teeth each. Six enamel slabs of equal size (about 2.5×2.5 mm) were cut from the approximal surfaces of each tooth (altogether 72 pieces) by using a water-cooled diamond saw. The outer enamel surface area of each slab was assessed by means of a precision micrometer (Tesa S.A., Renens, Switzerland). Afterwards, the slabs were rinsed with distilled water, dried, and mounted in dental wax, leaving only the enamel surface exposed. Six series made up of slabs from six different teeth were prepared within the two groups, and each series received identical treatment. One enamel piece in each series was not treated and served as a control. This experimental design was developed to eliminate intertooth variations and to provide comparisons among different treatments within the same tooth. For further details see Ref. 11.

Topical application of two fluoridated solutions for mouthrinsing (2 mg/ml and 0.5 mg/ml NaF), pH 5.5, of a commercial product (Fluorid, NAF-Laboratoriene A/S, Norway) was performed on enamel samples, using soaked standard-size cotton pellets (Cotton Pellets no. 4, Richmond Dental Co., Charlotte, N.C., USA), for 30 sec, 60 sec, 5 min, and 60 min. During the treatments all specimens were kept in closed plastic tubes. They were subsequently rinsed in distilled water while still positioned in the wax.

One slab from each treatment group was prepared for scanning electron microscopy (SEM), and the others were analyzed for fluoride on and in the enamel in accordance with procedures described by Saxegaard & Rølla (12).

Wilcoxon's matched-pairs signed test was applied to compare the amounts of alkali-soluble fluoride deposited on enamel after exposure to the fluoridated solutions for

mouthrinsing at different treatment times. The level of significance was set to $p < 0.05$.

Results

Table 1 shows the amounts of fluoride extracted from the enamel by alkali after the exposure to the fluoride solutions containing 0.2% NaF during 30 sec, 60 sec, 5 min, and 60 min. The amounts of fluoride increased with the time of exposure, but the differences were not statistically significant between treatments for 30 sec and 60 sec or for 30 sec and 5 min. The amount of alkali-soluble fluoride extracted after the fluoride treatment for 60 min was about 2.5 times higher than after 30 sec.

The results of the treatment of the enamel with a mouthrinse solution containing 0.05% NaF are shown in Table 2. Exposure to fluoride for 30 sec, 60 sec, and 5 min had no statistically significant effect on the amounts of fluoride formed. Only treatment for 60 min increased the deposition of alkali-soluble fluoride on the enamel. The amount was twice as high as after 30 sec.

Table 3 shows the results of the chemical analysis for firmly bound fluoride material from the samples treated for 60 min. There were no differences in fluoride uptake from

Table 1. Fluoride soluble in KOH from enamel surfaces of five teeth treated with a single topical application of a 0.2% NaF solution for mouthrinsing, at pH 5.5, during different periods of time

Variable	Weight of KOH-soluble F on enamel ($\mu\text{g} \cdot \text{cm}^{-2}$)		Matched comparisons†			
	Mean (n = 5)	SD	B	C	D	E
A	0.58	0.14	*	*	*	*
B	0.81	0.18	—	NS	NS	*
C	0.95	0.27		—	*	*
D	1.30	0.35			—	*
E	2.26	0.47				—

* Statistically significant difference. NS = no significant difference.

† A = control; B = 30 sec; C = 60 sec; D = 5 min; E = 60 min.

Table 2. Fluoride soluble in KOH from enamel surfaces of five teeth treated with a single topical application of a 0.05% NaF solution for mouthrinsing, at pH 5.5, during different periods of time

Variable value	Weight of KOH-soluble F on enamel ($\mu\text{g}\cdot\text{cm}^{-2}$)		Matched comparisons†			
	Mean (n = 5)	SD	B	C	D	E
A	0.61	0.17	NS	NS	NS	*
B	0.57	0.09	—	NS	NS	*
C	0.65	0.07	—	—	NS	*
D	0.70	0.13	—	—	—	*
E	1.19	0.24	—	—	—	—

* Statistically significant difference. NS = no significant difference.

† A = control; B = 30 sec; C = 60 sec; D = 5 min; E = 60 min.

the control group and the treatment groups; that is, no firmly bound fluoride was acquired by the enamel during exposure to fluoride solutions for mouthrinsing.

Fig. 1 illustrates the deposition of calcium fluoride-like material on enamel after a single topical application of a 0.2% NaF mouthrinse solution during different periods of time. Deposits of calcium fluoride-like material were formed in all cases, and an increase occurred with time of treatment. Granules were formed on the enamel surface (Fig. 1B) even during the shortest period of application. The micrographs also show that there is a tendency for agglomeration of the deposits. This effect was more evident during the 60-min treatment (Fig. 1E).

The scanning electron micrographs corresponding to the treatment with a 0.05% NaF mouthrinse solution during different periods of time are shown in Fig. 2. Precipitation of a granular layer of calcium fluoride on the enamel surface occurs in all cases. The amounts of the deposited material increase with the increase of the application time. The reaction was quite similar to the treatment with a more concentrated solution from a morphologic point of view. The 60-min treatment (Fig. 2E), however, promoted a more regular and uniform distribution of the globules over the surface of the sample.

Discussion

The present investigation has demonstrated that even short exposure times to a neutral 0.2% NaF solution result in deposition of alkali-soluble fluoride on the enamel. Treatment times from 5 to 60 min were used in the study to provide increased reactions and thus increase the sensitivity of the chemical analysis. The enamel samples did not have any pellicle material deposited on the surface, and it can thus be argued that this is an unphysiologic situation. However, it has been shown previously that fluoride penetrates the pellicle and forms the same amounts of calcium fluoride-like material on the surface as in samples without a pellicle (13).

Even the 0.05% NaF soluble induced formation of alkali-soluble fluoride on sound enamel in vitro, but measurable amounts were found only after a 60-min exposure. Thus the latter method appears to be more

Table 3. Firmly bound fluoride etched off from the enamel samples which received topical treatment with fluoridated solutions for mouthrinsing during 60 min

Treatment	Weight of F in enamel ($\mu\text{g}\cdot\text{cm}^{-2}$)		
	First layer	Second layer	Third layer
Control (no treatment)	0.10 ± 0.03	0.06 ± 0.02	0.06 ± 0.01
0.2% NaF	0.10 ± 0.02	0.06 ± 0.01	0.06 ± 0.008
0.05% NaF	0.10 ± 0.01	0.05 ± 0.005	0.05 ± 0.008

Mean (n = 5) ± SD.

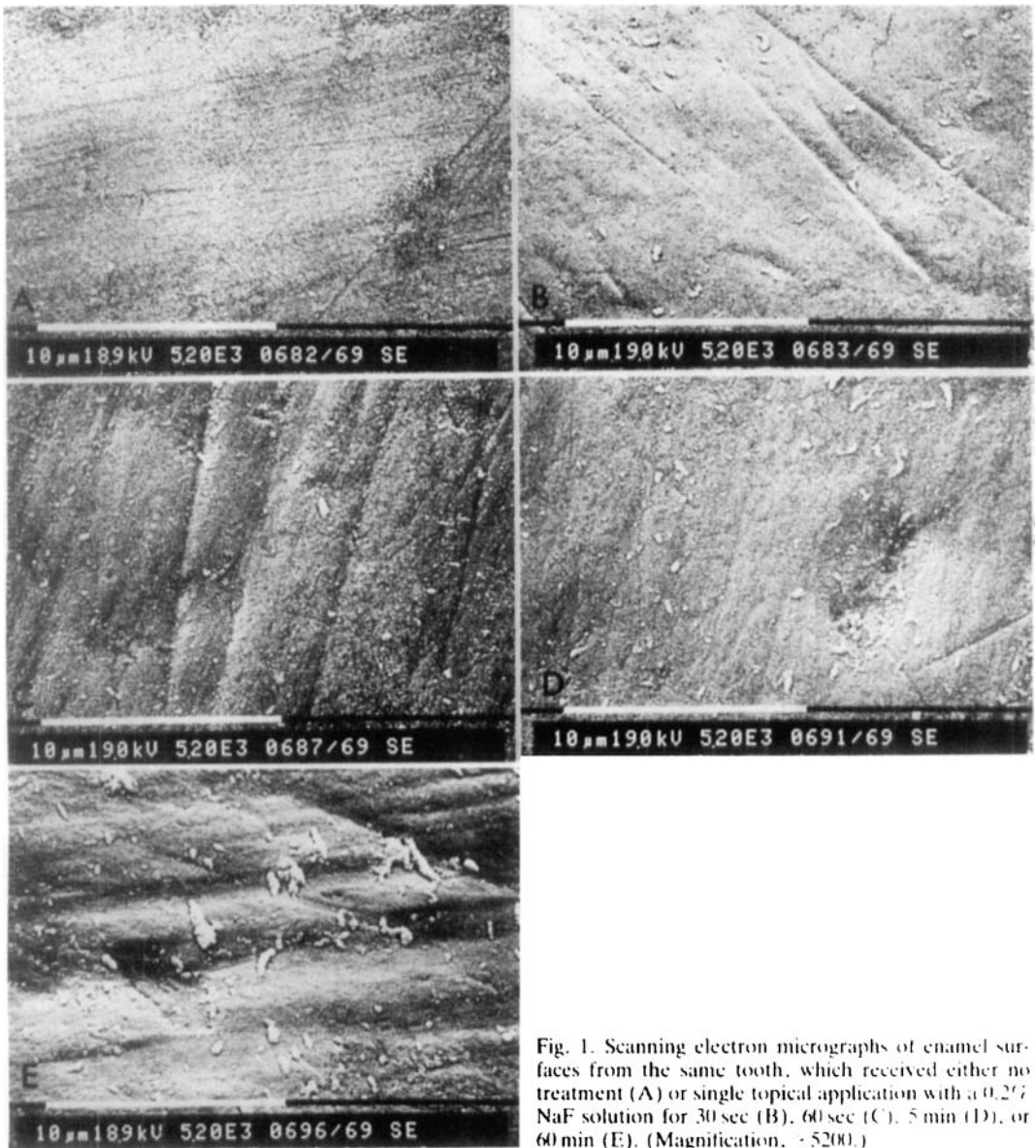


Fig. 1. Scanning electron micrographs of enamel surfaces from the same tooth, which received either no treatment (A) or single topical application with a 0.2% NaF solution for 30 sec (B), 60 sec (C), 5 min (D), or 60 min (E). (Magnification, $\times 5200$.)

sensitive than the former. The scanning electron micrographs showed deposits of a calcium fluoride-like material also after shorter exposures. The study thus supports the observations of previous *in vivo* investigations that fluoride mouthrinsing promotes precipitation of calcium fluoride-like

material on the enamel and in the plaque matrix (14, 15).

The present experiment was performed on enamel without any kind of visible defects. Most likely, demineralized enamel takes up more fluoride because acid calcium phosphates are better substrates for calcium flu-

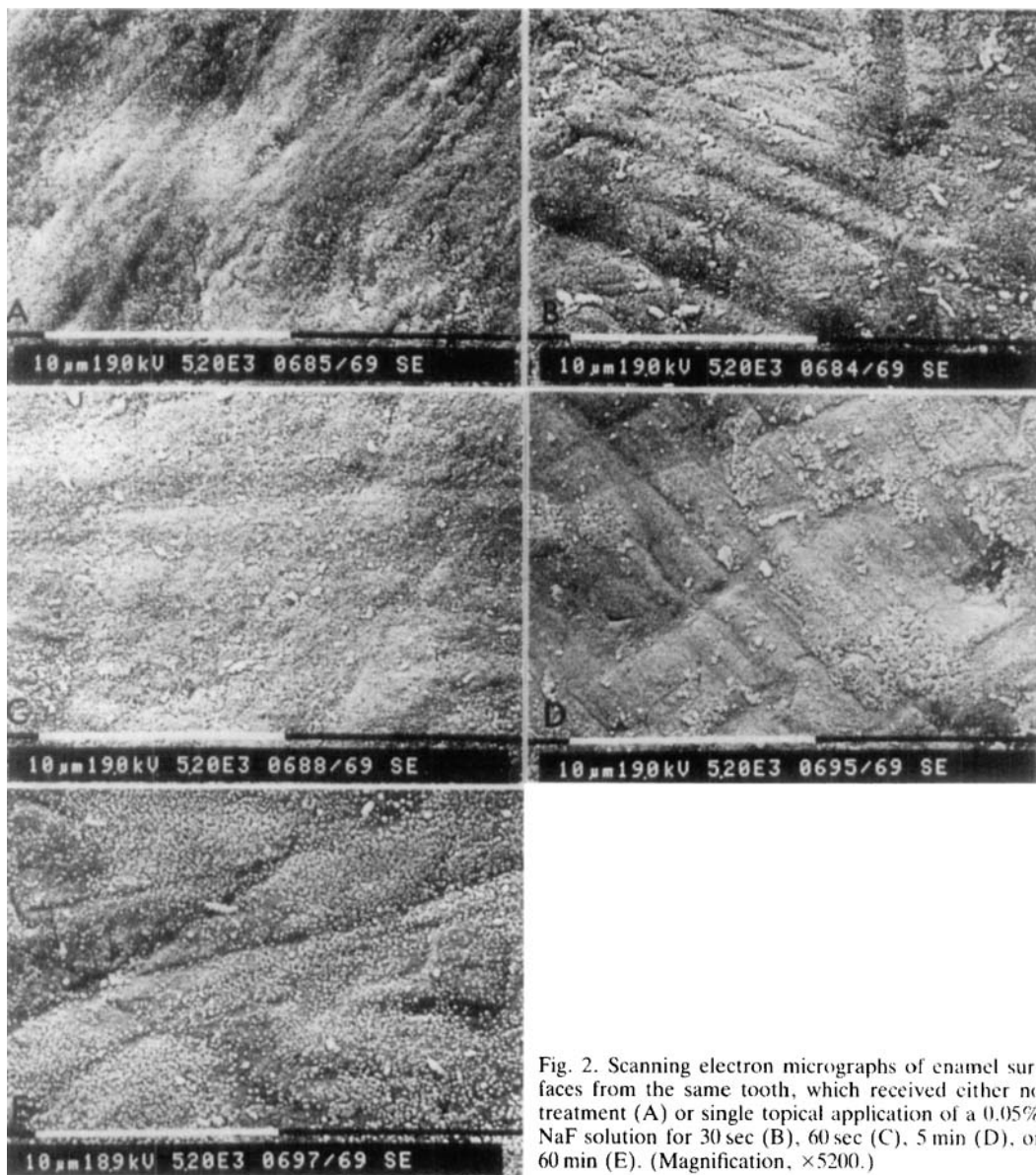


Fig. 2. Scanning electron micrographs of enamel surfaces from the same tooth, which received either no treatment (A) or single topical application of a 0.05% NaF solution for 30 sec (B), 60 sec (C), 5 min (D), or 60 min (E). (Magnification, $\times 5200$.)

oride formation than sound enamel (16). It appears that this may be the major mechanism by which 0.05% NaF works in vivo.

An important observation was that exposure of enamel to mouthrinse solutions did not give any incorporation of firmly bound fluoride (fluorhydroxyapatite or

fluorapatite). In a previous in vivo study (14) a slight increase in the firmly bound fluoride was observed after 2 weeks of rinsing with 0.05% or 0.2% NaF. It appears likely that deposition of a calcium fluoride-like material is an essential first step in the caries-preventive mechanism of topically applied

fluoride. Firmly bound fluoride is probably formed as a final product during demineralization/remineralization reactions, in which the fluoride ions originate from the calcium fluoride-like material (17, 18).

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