Alkali-soluble fluoride deposition on human enamel exposed to monofluorophosphate-containing toothpastes in vitro

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Enamel specimens from unerupted third molars were treated with the supernatant of monofluorophosphate (MFP)-containing toothpaste slurries in water. Calcium fluoride-like particles were formed on the enamel surface during a 24-h exposure. The globules were soluble in alkali. More particles were seen on enamel treated with a toothpaste containing both MFP and NaF. The experiments showed that the calcium fluoride-like material formed on enamel after treatment with MFP-containing toothpaste probably originates from the free fluoride present in the pastes. The relatively moderate deposition of fluoride may be of clinical significance because the teeth are so frequently exposed to toothpaste. No firmly bound fluoride was found on treatment of enamel with MFP-containing toothpaste. □ *Adsorbed fluoride; calcium fluoride; enamel surface; monofluorophosphate*

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Toothpastes are probably the most important fluoride-containing consumer product. A large number of clinical studies demonstrate a cariostatic effect of fluoride-containing toothpaste (1). Fluoride-containing toothpaste is considered the major factor responsible for the caries reduction observed in the Western world during the past few decades (1–3).

Toothpastes contain either monofluorophosphate (MFP) or NaF, or both, as a fluoride source. NaF provides free fluoride, whereas the covalently bound fluoride in MFP requires enzymatic hydrolysis to release free fluoride (4). This was clearly shown in an in vivo study in which no MFP could be detected in plaque-covered lesions as opposed to clean lesions (5). It has been demonstrated that the clinical effect of topical application of MFP and NaF solutions is similar (6, 7). They were also equally effective in depositing fluoride into artificial carious lesions in vivo (8).

The exact cariostatic mechanism of fluoride toothpaste is still debated. Some researchers assume that they induce the formation of fluorapatite or fluorhydroxyapatite in the enamel surface and that the reduced solubility causes the clinical effect. A more recent view is that a fluoridated toothpaste promotes deposition of soluble calcium fluoride-like and adsorbed fluoride on the enamel surface and in lesions (9–11). Calcium fluoride and adsorbed fluoride are soluble in alkali. It has been shown that calcium fluoride is quite stable in saliva owing to secondary phosphate adsorption. It has been suggested that calcium fluoride acts as a pH-controlled reservoir of fluoride ions on enamel or in plaque (12, 13).

The aim of the present investigation was to study the formation of alkali-soluble fluoride (calcium fluoride/calcium fluoride-like material and adsorbed fluoride) on application of MFP-containing toothpastes on human enamel surface in vitro.

Materials and methods

Experimental design

Surgically extracted impacted third molars were used in this investigation. After extraction they were cleaned and inspected in a stereomicroscope. Specimens with visible defects were discarded. Six blocks with an enamel surface measuring approximately 6.5 mm^2 were cut from the relatively flat approximal surfaces of each tooth with a water-cooled diamond-coated saw. The blocks were rinsed with distilled water, dried, and mounted in dental wax, leaving only the enamel surface exposed.

Topical treatment of the enamel surfaces was carried out by means of standard-size cotton pellets, soaked in supernatants from three different MFP-containing toothpastes (Colgate Classic F, Colgate-Palmolive A/S, Denmark; Pepsodent Fluor and Solidox Mint Fluor, A/S Denofa og Lilleborg Fabriker, Norway), at pH 7.0, for 24 h. All samples were kept in closed plastic tubes during treatment and were subsequently rinsed in distilled water while still mounted in wax. Colgate Classic F contained both NaF and MFP. Extracts from the different toothpastes were prepared by suspending 1 g of dentifrice in 3 ml distilled water. The suspensions were agitated for 1 min on a Vortex mixer, placed on a wrist action shaker for 5 min, and then centrifuged at 8000 *g* for 30 min at +4 °C. The presence of free fluoride in the supernatants was assessed with an ion-specific electrode (Orion Research, Cambridge, Mass., USA).

Series of 6 enamel blocks (36 blocks altogether), randomly selected from the different teeth, were assigned to the different treatment regimens. One series of each group was left untreated and served as a negative control, whereas a second series was treated with a neutral 2% NaF solution for 24 h, serving as a positive control. Other series were exposed to a 10 ppm fluoride solution at pH 7.0, for 24 h.

One block from each treatment group was prepared for scanning electron microscopy (SEM). They were dried and sputter-coated with a layer of gold-palladium and examined in a Phillips SEM-515, operated at 15.0-15.9 kV. For further details see Cruz et al. (10).

Analysis

The samples designated for chemical assessments were analyzed for alkali-soluble fluoride deposited on enamel by means of the method proposed by Caslavska et al. (14). The enamel blocks were placed in sealed plastic cups with 150 µl 1M KOH and gently agitated for 24 h at room temperature. Only the exposed enamel surface was subjected to KOH treatment. The solutions were neutralized with 150 µl 1M HCl, and 30 µl of TISAB III (Orion Research) was added. The fluoride concentrations were subsequently measured with a fluoride ion activity electrode connected to an EA 920 Expandable Ion Analyser (Orion Research).

The amount of alkali-soluble fluoride in micrograms was calculated by applying the equation $WCaF_2(\mu g) = 2.11\ WF$, in which WF is the weight (µg) of fluoride in the alkali solution. Chemical analysis for determination of firmly bound fluoride in enamel was also performed. After extraction of alkali-soluble fluoride, the blocks were rinsed in distilled water for 30 sec and air-dried at ambient temperature. A layer of nail varnish was applied, leaving only the enamel surface exposed, and three subsequent layers of enamel were etched off with perchloric acid (0.1 M). The dissolution was stopped by adding 0.1 M trisodium citrate. The ionic strength and pH were controlled with TISAB (Orion Research). The fluoride was analyzed by using the fluoride ion electrode and the amounts related to the area of each block.

These analytical procedures were applied as previously described elsewhere (10).

Statistical analysis

The Mann–Whitney two-sample rank test was used to compare the amount of alkali-soluble fluoride deposited on enamel after exposure to the supernatants from three different MFP-containing toothpastes and a solution containing 10 ppm F^-. The level of significance was set at 5%.

Table 1. KOH-soluble F from enamel surfaces of teeth treated with a single topical application of monofluorophosphate (MFP)-containing toothpastes or NaF solutions, at pH 7.0, for 24 h

Series	Treatment	Weight of KOH-soluble F on enamel ($\mu g \cdot cm^{-2}$)	
		Mean ($n = 5$)	SD
1	None	0.32	0.06
2	2% NaF	134.42	36.21
3	Colgate MFP + NaF	2.91	0.60
4	Pepsodent MFP	1.22	0.19
5	Solidox MFP	1.79	0.52
6	10 ppm F^-	1.35	0.53

Results

Chemical analysis of the supernatants demonstrated that Colgate, Pepsodent, and Solidox contained 140, 10, and 10 ppm F^- as free fluoride, respectively.

The results of the chemical assessments of alkali-soluble fluoride extracted from enamel surfaces treated with MFP-containing toothpastes for 24 h are presented in Table 1. Topical application of Colgate (which contained NaF in addition to MFP) formed about twice as much CaF_2 as the two pastes containing only MFP. This difference was statistically significant ($p < 0.05$). Topical application of an aqueous solution with 10 ppm of fluoride gave a deposition of calcium fluoride on the enamel surface of the same order of magnitude.

No firmly bound fluoride could be demonstrated (results not shown).

As expected, treatment of the enamel with a 2% NaF solution gave the highest value for deposition of calcium fluoride-like material.

The scanning electron micrographs of the treated enamel surfaces supported the chemical data. Fig. 1A shows the result of topical application of a 2% NaF solution for 24 h. A dense layer of large globules was deposited on the enamel surface. Fig. 1B shows a scanning electron micrograph from an enamel surface treated with Colgate (which contains both NaF and MFP). It can be seen that a layer of a granular deposit is present. The surface granules are not as densely packed as after the treatment with 2% NaF solution, most probably owing to the lower concentration of fluoride. The scanning electron micrographs obtained with the topical application of Pepsodent and Solidox are shown in Figs. 1C and 1D. Scattered particles can be seen on the enamel surface. In some areas aggregates of the deposited material were observed.

Fig. 1E shows the result of topical application of a neutral 10 ppm F^- solution for 24 h. Here, again, particles on the mineralized tissue can be seen. However, from a morphologic point of view, the appearance of the deposited material was not quite similar to that obtained with the application of supernatant from dentifrices. The layer is thinner, and the globules seem to be smaller.

Discussion

The KOH technique introduced by Caslavska et al. (14) enables loosely bound fluoride (calcium fluoride/calcium fluoride-like material and adsorbed fluoride) to be separated from firmly (apatitically) bound fluoride. By combining the KOH technique with scanning electron microscopy (SEM) (15), the present study clearly demonstrated that MFP-containing toothpastes induced formation of calcium fluoride-like material on enamel in vitro (Table 1, Fig. 1). The present study confirmed than MFP toothpastes contain a significant amount of free fluoride (16). Furthermore, MFP is not hydrolyzed in alkali (5). Accordingly, the formation of calcium fluoride-like material observed was most likely due to the presence of free fluoride in the MFP (only) toothpastes, since a 10-ppm fluoride solution gave deposition of the same amounts of calcium fluoride (Table 1, series 6, and Fig. 1E).

No measurable increase in the firmly bound fluoride was found after treatment with any of the toothpastes, supporting a previous study (10). The increase in firmly bound fluoride observed after topical fluoride treatment is thought to be due to a secondary reaction during pH-cycling in plaque (12, 13).

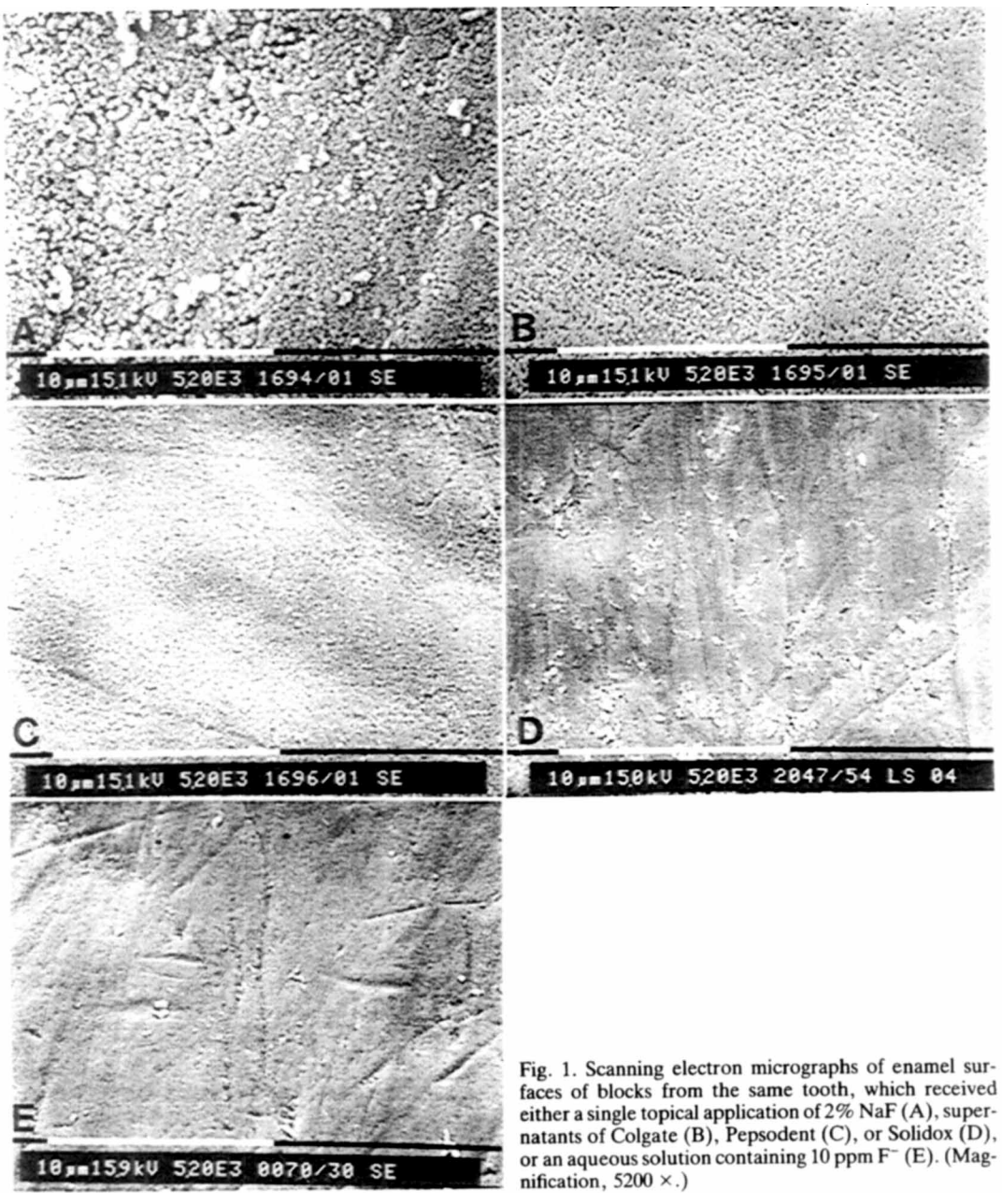

Fig. 1. Scanning electron micrographs of enamel surfaces of blocks from the same tooth, which received either a single topical application of 2% NaF (A), supernatants of Colgate (B), Pepsodent (C), or Solidox (D), or an aqueous solution containing 10 ppm F^- (E). (Magnification, 5200 ×.)

The enamel samples used in the present study were obtained from unerupted teeth. These are more reactive than erupted teeth owing to their content of carbonate apatite. However, since the major source of substrate for calcium fluoride formation is demineralized enamel (17), the use of unerupted teeth may be considered a better model than erupted teeth with an unknown history. The present experimental design, in which the toothpastes were all tested on samples from the same tooth (with five parallels) enables

conclusions to be drawn even though the variations in reaction pattern among the individual teeth is relatively large (observe the standard deviations in Table 1). It can also be argued that incubation of the teeth for 24 h with the toothpaste supernatants is a relatively long incubation time. This time was chosen for analytical reasons, to obtain fluoride concentrations in the alkali extract which were well above the detection limit. The results indicate that calcium fluoride, albeit in smaller amounts, will be formed at certain locations on the tooth surface even when shorter exposure times are used.

It should be understood that additional fluoride becomes available from MFP paste in vivo because of hydrolysis of MFP.

If clinical implications should be drawn on the basis that far less fluoride is retained on the enamel samples treated with toothpaste than with those treated with 2% NaF, it should be borne in mind that toothpaste is used once or twice daily, whereas 2% NaF is applied every 3rd or 6th month.

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