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Original scientific paper

Effect of Amine Fluoride on Enamel Surface Morphology

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A B S T R A C T

In this in vitro study, examination of the enamel surface morphology after topical application of an amine fluoride solution with different fluoride (F) content was carried out. Sound human enamel slabs were treated with an amine fluoride solution containing either 1.0, 0.5 or 0.25% F for 3 min. during 3 days. All slabs were examined using scanning electron microscopy and energy-dispersive spectroscopy (SEM/EDS) qualitative analysis. The globular precipitates were revealed in all treated specimens, regardless of F content. The distribution of the deposits was more homogeneous in groups treated with higher concentrations; however, the globules were larger and more cubical in groups treated with lower fluoride concentrations. These larger globules could be less soluble and thus serve as a fluoride reservoir for a more extended period and so they could contribute to the caries preventive effect in professional topical products with lower fluoride concentration. Following the 24-hour treatment with KOH the precipitates could be removed; however, the enamel surfaces covered with the precipitates were less degraded than the untreated enamel. The EDS qualitative analysis showed that the intensities of fluoride signals were increased with the higher concentration of fluoride in an amine fluoride solution, while the intensities of calcium signals were decreased. The enamel surface precipitates were alkali-soluble, but we were not able to demonstrate that they are pure calcium fluoride.

Introduction

In a large number of studies, professionally applied topical fluorides have been shown to be effective in caries prevention. However, the exact mechanism by which fluoride causes caries prevention (»mode of action«) has not been completely understood. In recent years, it has been suggested that calcium fluoride (CaF_2) or calcium fluoride-like material is the main product deposited on enamel after exposure to high concentrations of fluoride and is most likely essential to the

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cariostatic mechanism of topical fluoride[1–7]. Furthermore, it has been suggested that CaF_2 may serve as a source of ionic fluoride (F). During the cariogenic challenge, CaF_2 releases F ions that are subsequently incorporated into enamel as fluorhydroxyapatite (FHAP) or fluorapatite (FAP)[1,4].

The condition by which calcium fluoride formation on the teeth was affected has been examined recently. Extended exposure time, increased fluoride concentration in the applied solution or a lowered pH caused increasing deposition of calcium fluoride on the enamel[8]. However, topical fluoride preparations are formulated at different concentrations of fluoride and a dose/response relationship to fluoride does not exist in agents for professional application[5].

The reaction of topical fluoride to the enamel surface has been explored using chemical assay[9,10], electron microprobe and x-ray diffraction techniques[11], scanning electron microscopy[12–16] and other methods[17–20].

The morphological appearance of the deposits on the enamel surface in the scanning electron microscope (SEM) after exposure to fluoride has been variously described. Microcrystals and amorphous coating have been observed. The deposits are mostly described as spherical globules[21]. Some authors speculated that phosphate is responsible for the globular structure, as pure calcium fluoride is cubical[22].

In order to better understand the mechanism of the anticaries effect of the surface deposits and their possible influence on a dose/response relationship in fluoride professional products, an attempt was made in the present study to examine the enamel surface micromorphology after topical application of an amine fluoride solution with different fluoride content under in vitro conditions. Scanning electron microscopy (SEM) was used for examination of possible morphological changes occurring on the tooth surface, and energy-dispersive spectroscopy (EDS) qualitative analysis was used for determination of the elemental composition of the surface deposits.

Materials and Methods

Ten impacted human third molars with clinically sound enamel were provided by oral surgeons and stored in a humid environment until used. Then, they were brushed with non-fluoridated pumice powder and water. The buccal or lingual surfaces were examined under a magnifying glass (10 magnification). Only surfaces without any visible defects were selected for the treatment on 3 days with an amine fluoride solution for 3 min. Four enamel slabs of about 4 5 mm were cut from each of 10 teeth by use of a water-cooled diamond wheel. After cutting, all slabs were rinsed with distilled water and four groups of enamel slabs were selected at random so that one slab from each tooth was present in each group. One group of slabs was not treated and served as control. Each of three groups of the slabs was immersed for 3 min. in an amine fluoride solution (pH 3.8) (Aminfluorid otopina®, Belupo, Croatia; N, N, N' -3-bihydroxyethyl-N'-octadecyl-1,3-diami nopropan-dihydrofluoride) at room temperature with one of the following fluoride concentrations: (a) 1% F, (b) 0.5% F and (c) 0.25% F. Subsequently, the specimens were rinsed in distilled water for 30 s and then were kept in closed plastic bags. These treatments were repeated for 3 days.

Two slabs from group (a) (1% F) were rinsed with distilled water for 30 s and soaked in test tubes containing 10 ml of 1 M potassium hydrochloride (KOH) for 24 h and 2 slabs from control (untreated) group were also exposed to KOH. This method was developed to identify the presence of CaF_2 precipitates on enamel specimens as described by Caslavska et al.[9]. Subsequently, the slabs were rinsed with distilled water for 30 s and were air dried at room temperature.

All specimens were then vacuum coated with highly conductive layer of gold palladium, approximately 500 Å, and enamel surfaces were viewed with a JOEL JSM-5 800 SEM, operating at 20 kV. The microscope was equipped also with an Oxford Instruments ISIS 302 EDS- qualitative analysis system. The enamel samples were analysed for the presence of F and Ca as described by Duschner et al.[20].

Results

Scanning Electron Microscopy

The morphologic appearance of the enamel surfaces in SEM revealed the presence of precipitates in all treated specimens. The precipitates varied in appearance. Amorphous, globular and crystalline structures were seen on the enamel surfaces following application any of three different amine fluoride concentrations. A distinct difference was observed between the treated and control groups (Figure 1). The precipitates did not cover the surfaces completely in any of the ca-

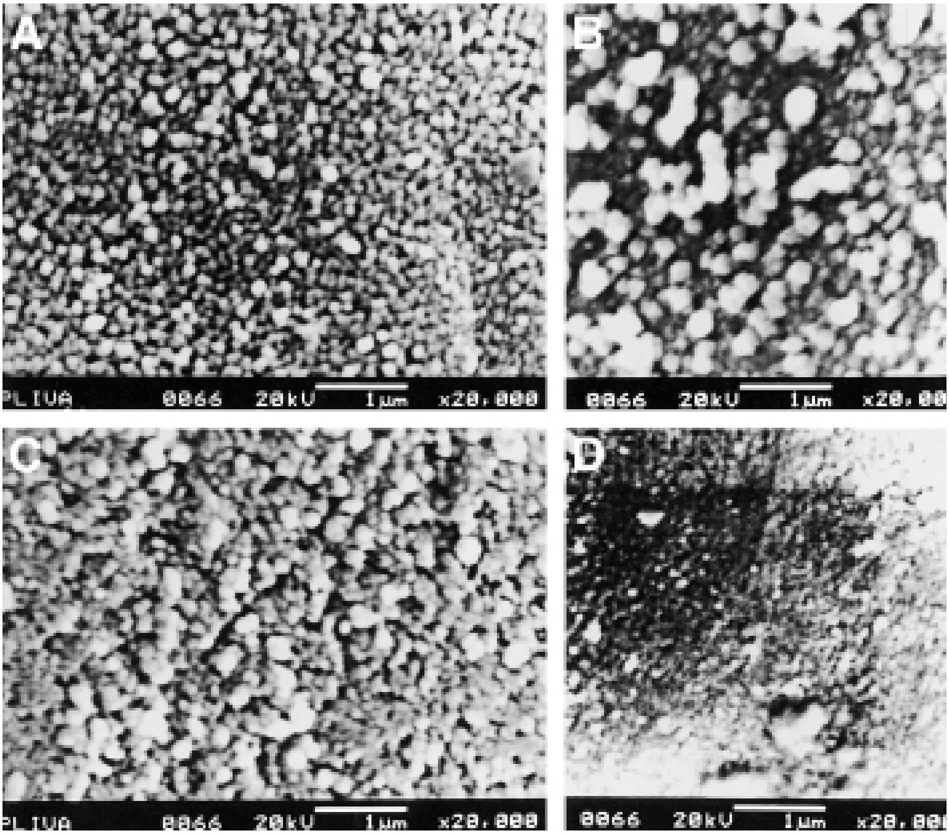

*Fig. 1. Scanning electron micrographs of sound enamel surfaces from the same tooth treated with different concentrations of an amine fluoride solution: 1% F (**a**), 0.5% F (**b**), 0.25% F (**c**) or not treated (control) (**d**).*

ses. Density of the deposits on the enamel surface increased when the higher a concentration of fluoride was used (Figure 1a, b). The deposits were spherical or globular in morphology, regardless of F concentration. The individual globules aggregated into larger particles up to 1 m in diameter. The deposits observed on the enamel samples treated with lower amine fluoride concentrations (0.5 and

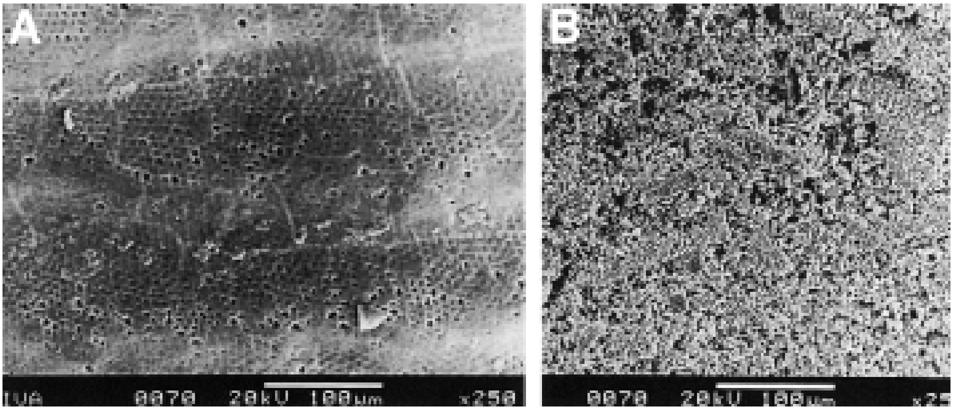

*Fig. 2. Scanning electron micrographs of enamel surface treated for 3 min. with an amine fluoride solution with 1% F (**a**) or not treated (**b**) and after KOH extraction.*

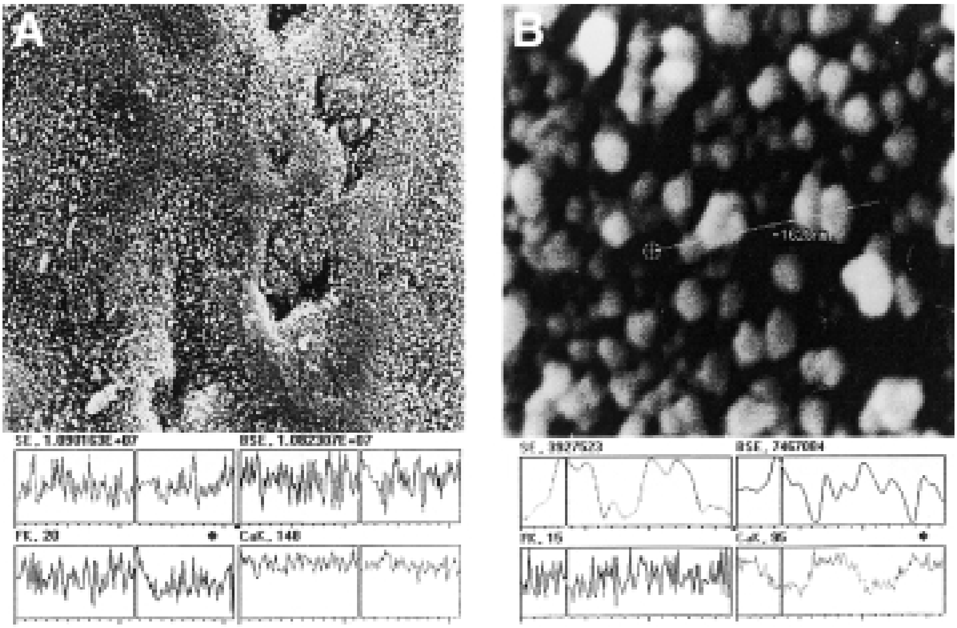

*Fig. 3. SEM images and EDS pattern of the enamel surface after application of an amine fluoride solution with 1% F (**a**) or 0.5% F (**b**). Above: SEM images. Below: Elemental line scans of the intensities of the F and Ca peaks.*

0.25% F) were less numerous but larger and in some cases of more cubical shape.

The KOH-extracted enamel surfaces are shown in Figure 2. The slabs were immersed in the KOH solution for 24 h after exposure to the 1% amine fluoride solution and considerable etching at the prism level was revealed. The granular surface deposits were removed by the KOH treatment. The enamel surfaces from the untreated group were severely e tched. The diffuse irregular type of etching was seen. The prism cores were more dissolved, while the interprismatic substance was less affected.

Energy-Dispersive-X-ray-Spectroscopy (EDS or EDX)

The energy-dispersive-spectrum qualitative analysis indicated the presence of Ca and F on all treated samples. Ca was the predominant element on the surfaces of all samples. The fluoride signal was present in all samples treated with amine fluoride solution. The intensities of the fluoride signals showed a tendency to increase on samples treated with higher fluoride concentrations. However, the intensities of the signals were very similar on the samples treated with 0.5 and 1% F amine fluoride solution (Figure 3a, b). Secondary electrons were recorded and they gave the topographical locations of the individual globules only in some samples (at magnification 5,000). In these samples a topographic discrimination of the EDS signals coming from the precipitates and from the underlying enamel was very good (Figure 3b). The line scans over selected area were characterised by decreases of the calcium signal in the correlation with the topographical position of the individual globules. The elemental composition of individual globules could not be obtained by the EDS scans when the globules were of small size. In the integral EDS spectra of the enamel surface, the signals of phosphorous and oxygen were found to be increased in correlation with the position of the individual globules (Figure 4). The results of the samples derived from the same tooth are also expressed quantitatively (the data from Link ISIS, version 3.2 computer program) and they showed that the percentage of F was similar (between 30 and 40 wt%) in samples treated with either a 0.5 or 1.0% amine fluoride solution. On the other hand, the percentage of Ca was decreased when concentration of F was increased. The percentage of Ca was similar (between 60 and 70 wt%) for samples treated with 0.5 or 1.0% F. The Ca:F ratios (by weight) were above 1.8 being more than theoretical ratio for CaF_2 (1:1 by weight). In the control, non-treated samples the percentage of the element F was below the limit of detection for this technique.

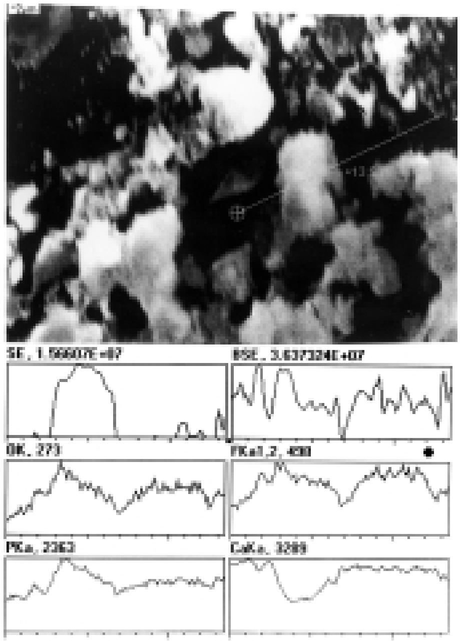

Fig. 4. SEM image and EDS pattern of enamel surface after application of an amine fluoride solution (1% F). Above: SEM image. Below: Elemental line scans of intensities of F, Ca, O and P peaks.

Discussion

This study confirmed that the morphology (SEM) of the enamel surface is changed by in vitro treatments with amine fluoride solution. The globular precipitates and amorphous structures were observed on the enamel surfaces under the conditions of this study. These precipitates are generally described by other authors as KOH – soluble fluoride or calcium fluoride-like material[9,22]. KOH treatment in our study removed most of deposits leaving an etched surface.

In some earlier studies the presence of the globular products on the enamel was also described after the short time of contact with topical fluoride agents[13,16,23,24]. However, Harding et al.[25] could not demonstrate the formation of calcium fluoride during clinically relevant treatment time (for 5 min or less) using neutral fluoride solution. The composition of the fluoride agent can have an effect on the production of CaF_2. Thus, the conventional 5-min treatment with NaF solution has produced the same amount of CaF_2 in caries-like lesions as an 18 hours exposure to Duraphat varnish[26]. Previous electron-spectroscopic study clearly demonstrated that the amount of fluoride deposited on enamel surfaces is mainly a function of the pH of the agent applied[27]. Each reduction in pH of topical solution resulted in a significant increase in the amount of alkali soluble fluoride[8]. Amine fluoride which was used in this experiment is an example of an acidic fluoride solution (pH 3.8).

The distribution of the globules was more homogeneous in the groups treated with a higher concentration of fluoride (1% F). This is similar to the results of an earlier study on the globular deposits of »calcium fluoride« on enamel[8].

Following the application of the lower fluoride concentrations (0.5 and 0.25% F) the sizes of globules were larger or more cuboidal in shape. The diameter of the globules is assumed to be influenced by the chemical properties of the applied fluoride solutions, especially the tensity of some amine hydrofluorides[28]. However, we used only one amine fluoride and the size of the globules could not be related to their chemical properties.

Nelson et al.[23] suggested that the particle size of CaF_2 crystallites may be an important factor in determining the effectiveness of a topical fluoride agent. It is well known that for particles in the nanometar range, the solubility of crystals decreases with an increase in size. Thus, the smallest particles would be more soluble and reactive than larger crystals of CaF_2. Therefore, we may assume that larger globules observed after treatment with low fluoride concentrations will dissolve more slowly and serve as a fluoride reservoir for more extended periods of time and so contribute to caries preventive effect. In our earlier clinical studies[29,30], lower fluoride concentration of amine fluoride solution (0.5% F) provided a similar caries preventive effect as a standard amine fluoride solution with 1% F did. Also, in some other clinical studies[31,32] no dose/response relationship was confirmed. Intra-oral caries model experiments with severe caries challenges showed also no dose/response relationship to the concentration of fluoride in the solutions[33].

Calcium fluoride formed on the tooth surface after topical application of fluoride is contaminated with phosphate and the amounts of phosphate adsorbed on the surface or incorporated into the calcium fluoride crystal are dependent on the conditions under which they have been formed. At high concentrations of fluoride or at low pH, calcium fluoride contains less internal phosphate and is less soluble than calcium fluoride formed under other conditions[4]. The EDS-qualitative analysis in this experiment gave evidence for the formation of deposits containing phosphate. So, their influence on the solubility of the deposits could not be excluded.

In our study the EDS qualitative analysis did not give clear evidence for the presence of CaF_2 globules. The intensity of calcium signals decreased in correlation with the topographical position of the individual globules. Obviously, the high intensity calcium signals between globules came from the underlying enamel and the globules shielded them. The intensity of fluoride signals was higher in groups treated with higher amine fluoride concentrations (0.5 and 1% F) than those treated with lower fluoride concentration (0.25% F). The Ca: F ratios (by weight) significantly above 1 give no evidence for the presence of pure calcium fluoride. The increased signals of phosphorous and oxygen in the position of individual globules could be interpreted as evidence for phosphate present in the globules. The experiments in vitro clearly showed that phosphate severely inhibits dissolution of CaF_2. Thus, Christoffersen et al.[22] suggested that pure CaF_2 crystals precipitated on dental enamel could not act as a slow fluoride release device. The calcium fluoride like materials containing phosphate appear to be more likely candidates to serve this purpose. So, »calcium fluoride-like« globules formed at lower concentrations of fluoride could contain more phosphate and serve as a fluoride long lasting depot. This could be an explanation for the beneficial effect of topical fluoride preparations with lower fluoride concentrations. Forms of reaction products other than CaF_2 could not be excluded as well. White et al.[19] used nuclear magnetic resonance techniques and revealed a new »reaction product« of fluoride/enamel interactions, designated as non-specifically-adsorbed fluoride (NSAF). NSAF is described as fluoride, which is hydrogen-bonded to the phosphate protons on the apatite. Those authors suggested that topical fluoride prevents caries primarily through the development of increased acquired acid resistance (AAR) in initial caries lesions and that NSAF species may have an important contribution to anticaries reactivity.

In conclusion, it may be stated that the amounts and the sizes of the globules deposited on dental enamel vary according to the fluoride concentrations. The fluoride concentration can influence on different dissolution properties of calcium fluoride[22] and be of clinical significance.

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UČINAK AMINOFLORIDA NA MORFOLOGIJU POVRŠINE CAKLINE

S A Ž E T A K

U ovoj je in vitro studiji istražena morfologija površine cakline poslije topikalne primjene aminofluoridne otopine s različitim sadržajem fluorida (F). Pločice, zdrave ljudske cakline obrađene su aminofluoridnom otopinom s 1.0, 0.5 i 0.25 % F u vremenu od 3 minute, tijekom 3 dana. Sve pločice su pregledane uporabom elektronske mikroskopije i kvalitativnom raščlambom spektra rasprostiranja energije (SEM/EDS). Na svim obrađenim uzorcima zapažene su kuglaste nakupine, bez obzira na sadržaj fluora. Raspodjela naslaga bila je jednakomjernija na uzorcima obrađenim s većim koncentracijama. Međutim, kuglice su bile veće i kockastije u skupinama obrađenim s manjim koncentracijama fluorida. Ove veće kuglice mogle bi biti slabije topljive i tako služiti kao spremište fluorida kroz duže vremensko razdoblje. Tako bi mogle pridonijeti karijes-preventivnom učinku proizvoda s manjim sadržajem fluorida namijenjenih lokalnoj stručnoj primjeni. Naslage su se mogle odstraniti nakon 24 sata obrade s KOH. Međutim, površine cakline prekrivene naslagama bile su manje razorene u usporedbi s kontrolom. EDS kvalitativna raščlamba je pokazala da su intenziteti signala fluora veći u caklini tretiranoj s aminofluoridnom otopinom veće koncentracije fluorida, dok su intenziteti signala kalcija manji. Nakupine na površini cakline topljive su u alkalijama, ali nismo mogli dokazati da su čisti kalcij fluorid.