Abstract

Introduction: Continuous balanced demineralization and remineralization are natural dynamic processes in enamel. If the balance is interrupted and demineralization process dominates, it may eventually lead to the development of carious lesions in enamel and dentine. Fluoride helps control decay by enhancing remineralization and altering the structure of the tooth, making the surface less soluble. Methodology: One hundred and twenty sound human permanent incisors randomly and equally distributed into six groups as follows: Group I - Control, II - Sodium fluoride solution, III - Sodium fluoride gel, IV - Sodium fluoride varnish, V - Clinpro Tooth Crème (3M ESPE), and VI-GC Tooth Mousse Plus or MI Paste Plus. The samples were kept in artificial saliva for 12 months, and the topical fluoride agents were applied to the respective sample groups as per the manufacturer instructions. Scanning electron microscope (SEM) evaluation of all the samples after 6 and 12 months was made. Results: Morphological changes on the enamel surface after application of fluoride in SEM revealed the presence of globular precipitate in all treated samples. Amorphous, globular, and crystalline structures were seen on the enamel surface of the treated samples. Clear differences were observed between the treated and untreated samples. Conclusion: Globular structures consisting of amorphous CaF$_2$ precipitates, which acted as a fluoride reservoir, were observed on the enamel surface after action of different sodium fluoride agents. CPP-ACPF (Tooth Mousse) and Tricalcium phosphate with fluoride (Clinpro tooth crème) are excellent delivery vehicles available in a slow release amorphous form to localize fluoride at the tooth surface.

Keywords: Clinpro tooth Crème (3M ESPE), GC Tooth Mousse Plus or MI Paste Plus (CPP-ACPF), scanning electron microscope, sodium fluoride

Introduction

In newly industrialized countries, the incidence of caries increases when people switch from a dependence on traditional starchy, staple foods to that on refined carbohydrates.[1]

Topical fluorides are widely used in pediatric dentistry as caries preventive agents.[2] Results of numerous clinical investigations clearly demonstrate the cariostatic effect of fluoride compounds in various forms of application both in the pre- and post-eruptive phases of tooth development.[3]

The purpose of this study is to compare the morphologic features of the enamel surface after the application of different topical fluoride products using scanning electron microscope (SEM).

Methodology

One hundred and twenty sound human permanent incisors extracted due to periodontal reasons were selected for the study in the Department of Pediatric Dentistry and Department of Biochemistry, Genesis Institute of Dental Sciences and Research, Ferozepur, after obtaining the ethical clearance from the Baba Farid University of Health Sciences, Punjab, in association with the Department of SEM, IIT, Delhi [Figure 1].

Preparations of specimens

The radicular part of each tooth was removed with the help of diamond disc mounted on straight handpiece [Figure 2]. The coronal part thus obtained was then brushed for 60 seconds with detergent to remove organic matter followed by thorough washing in water. An acid resistant
nail varnish was applied on all the surfaces except on the enamel surface. The samples are then randomly divided into six groups \((n = 20)\) and stored in artificial saliva. The topical fluoride agents were applied to the respective sample groups, followed by washing in distilled water for 30 seconds to remove unreacted fluoride agent. Half of the samples from each group were analyzed under SEM at 5000x and 25000x magnification for all the six groups after 6 months and rest of the samples after 1 year.

**Preparation of artificial saliva**

The artificial saliva was prepared according to Macknight-Hane and Whitford (1992) formula.

**The composition of artificial saliva (g/L)**

- Methyl-p-hydroxybenzoate \(2.00\)
- Sodium Carboxymethyl Cellulose \(10.00\)
- KCl \(0.625\)
- MgCl\(_2\) 6H\(_2\)O \(0.059\)
- CaCl\(_2\) 2H\(_2\)O \(0.166\)
- K\(_2\)HPO\(_4\) \(0.804\)
- KH\(_2\)PO\(_4\) \(0.326\)

The pH of artificial saliva was adjusted to 6.75 with addition of KOH [Figure 3].

**Procedure**

The coronal portion was subjected to the following surface treatments.
- **Group I - Control \((n = 20)\):** The specimens were washed with distilled water and placed in a clean container containing artificial saliva
- **Group II - Sodium fluoride solution \((n = 20)\):** Specimens were prepared by applying a freshly prepared solution of 2% sodium fluoride four times a year at weekly interval for 4 min, followed by washing with distilled water and stored in artificial saliva
- **Group III - Sodium fluoride gel \((n = 20)\):** Semi-annual application of commercially obtained sodium fluoride gel for 4 min
- **Group IV - Sodium fluoride varnish \((n = 20)\):** Semi-annual application of commercially obtained sodium fluoride varnish for 4 min
- **Group V - Clinpro Tooth Creme (3M ESPE) \((n = 20)\):** Specimens were prepared by carrying out a daily application of commercially obtained Clinpro Tooth Creme for 4 min
- **Group VI - GC Tooth Mousse Plus or MI Paste Plus \((n = 20)\):** Daily application of commercially obtained GC Tooth Mousse Plus for 4 min.

**Scanning electron microscope sample preparation**

SEM specimens were attached to aluminum stubs with a fast-curing epoxy resin [Figure 4]. The samples were then sputtered with a 10–12 nm thick coating.
layer of gold in a Balzer-Union sputter apparatus [Figures 5 and 6]. The specimens were examined under a low and high (×5000 and ×25,000) magnification in a SEM operated at 20 kv. The results were recorded by photo micrographic techniques and evaluated after 6 months and rest of the samples after 1 year.

Results

Several morphological changes on the enamel surface were seen after application of different sodium fluoride agents in SEM. It revealed the presence of globular precipitate in all treated samples.

Untreated anatomical surfaces

**Group I - Control**

The surface of the untreated enamel slab at 6 and 12 months appeared smooth and homogenous. In some of the samples, few surface depressions or irregularities were visible which could be due to the polishing of the enamel slabs [Figure 7].

Treated anatomical surfaces

Distinct surface coating deposited by all five of the topical fluoride agents were evident on the anatomical enamel surfaces of the specimens. However, different morphology was observed with different topical sodium fluoride agents.

**Group II - Sodium fluoride solution**

When samples coated with sodium fluoride solution were viewed at 6 months, entire enamel surface was covered with surface reaction product. Areas of calcified deposits were evident. The circular globules of calcium fluoride were observed in the reaction product layers produced by the sodium fluoride solution application [Figure 8a]. The globules were almost even in size and ranged from 0.2 to 0.6 µm. They were scattered homogenously on the enamel surface with little tendency to form agglomerates. When samples were viewed after 12 months, not much difference was seen, as no additional fluoride application was done on the enamel surface after 6 months [Figure 8b]. When the sodium fluoride solution was compared with other materials, the size of the calcium fluoride crystal formed was larger in size except the sodium fluoride varnish in which size of the crystal was greater and more agglomerates of the crystals were formed.

**Group III - Sodium fluoride gel**

When samples coated with sodium fluoride gel were viewed at 6 months, the porosities were evident on the enamel surface and faint lines of mineralization could be seen in and around the porosities. Areas of mineralized deposits in the form of spherical globules agglomerates which were formed of calcium fluoride were discernible and seen profusely scattered along the porous defects. The size of the crystal formed ranged from 0.05 to 0.2 µm which was of very small size as compared to other materials [Figure 9a]. After the second application, at 12 months, enamel surface was homogenously covered with spherical globules which varied in size from 0.1 to 0.2 µm [Figure 9b].

![Figure 5: Balzer-Union sputter apparatus](image)

![Figure 6: Gold-sputtered enamel slab](image)

![Figure 7: Scanning electron microscope image of untreated intact human enamel surfaces at 6 months and 12 months](image)

![Figure 8: SEM image of enamel surfaces treated with sodium fluoride solution at 6 and 12 months (Mag 25.00 KX, WD =11 &10mm)](image)
Group IV - Sodium fluoride varnish

The Varnish produced the most distinctive surface layer morphology. When samples were viewed at 6 months, the entire enamel surface was covered with surface reaction product, spherical globular agglomerates of calcium fluoride were observed in the reaction product layers. The roughly spherical globules varied in size from 0.2 to 1 µm on the enamel surface. The spherical globules appeared to coalesce and form a surface layer microstructure. The large clumps of reaction products on the outer surface of the coating were often seen to consist of this microstructure [Figure 10a]. When the rest of the samples were viewed at 12 months, more concentrated spherical globules were seen, and the size of the crystal formed ranged from 0.1 to 0.5 µm [Figure 10b]. This group showed the best surface morphology when compared with other groups as the calcium fluoride crystal formed in this group were of maximum size and the agglomerates formed by these crystal covered maximum enamel surface.

Group V - Clinpro Tooth Crème (3M ESPE)

When the samples were viewed at 6 months, spherical globular agglomerates of calcium fluoride were observed in the reaction product layers produced by the clinpro tooth crème application. The roughly spherical globules varied in size from place to place on enamel surface and ranged in size from 0.08 to 0.4 µm. The spherical globules appeared to coalesce and form a surface layer microstructure. These globules also appeared to consist of agglomerates of still smaller particles with a size of about 30 nm in diameter. However, the surface coating was not entirely covered with globules; only the small area on the enamel surface was evident with clumps or mounds of spherical globules while much of the enamel surface was rough without any globular structure [Figure 11a]. The rest of the samples when viewed at 12 months, the entire enamel surface was covered with surface reaction product [Figure 11b]. Spherical globules were observed ranging from 0.1 to 0.2 µm which was of smaller size as compared with sodium fluoride solution and varnish but comparable in size with that of GC Tooth Mousse Plus.

Group VI - GC Tooth Mousse Plus or MI Paste

The concentration of fluoride in GC Tooth Mousse Plus or MI Paste and Clinpro tooth crème is same, but the reaction products formed by both are different. When sample was viewed at 6 months, spherical globular agglomerates were observed in the reaction product layers produced by the GC Tooth Mousse Plus application. The spherical globules agglomerates varied in size from 0.1 to 0.2 µm. These agglomerates were formed from smaller globules which were approximately 30 nm in diameter. However, the surface coating was not entirely covered with globules, and regions of the surface coating with no visible spherical globules between the large surface clumps or mounds were evident [Figure 12a]. The rest of the samples, when viewed at 12 months, showed spherical globules uniformly scattered all over the enamel surface ranging in size from 0.2 to 0.3 µm [Figure 12b]. Under higher magnification, surface coating formed by spherical globules showed agglomerates which were distributed all over the enamel surface. When compared with Clinpro Tooth Crème, the surface area covered by the spherical globules was more in GC Tooth Mousse Plus and the agglomerates of calcium fluoride globules formed on the enamel surface were denser. The spherical globules were comparable in size with sodium fluoride gel but smaller than sodium fluoride varnish and solution.
Discussion

In the past, it was believed that the relationship between the use of fluoride by systemic administration and the significant reduction of caries was due to its incorporation during enamel formation. Thus, the main objective of the therapy was to promote an increase of incorporation to its structure. Low and constant concentrations of fluoride in the oral cavity are more efficient for disease control.

An important factor to consider is the particle size of the surface coating. In the present study, SEM pictures revealed that the smallest particles were approximately 30 nm in diameter. However, these particles were covered on both sides with a 10–12-nm-thick layer of gold, which means that the smallest CaF₂ particles were in the 5–10-nm range. It is well known that, for particles of such a small size, the solubility of crystals decreases with increasing size, as described by the following equation:

$$\ln \frac{K_{sp}(r)}{K_{sp}(\infty)} = \frac{2MY_{sl}}{RT}\rho r$$

Where $K_{sp}(r)$ represents the solubility of spherical crystals of radius, $r$, $K_{sp}(\infty)$ is the solubility product of large crystals, $Y_{sl}$ is the surface free energy for the solid-liquid interface, $\rho$ is the density of the material, $M$ is the formula weight, $T$ is the absolute temperature, and $R$ is the gas constant (8.3143 J/K/mol).

In the present study, when the morphology of the sound enamel surface and the particle size was observed after the application of different sodium fluoride groups, it was seen that best results were shown by sodium fluoride varnish followed by sodium fluoride solution. These were followed by GC tooth mousse, Clinpro tooth cream, and sodium fluoride gel whereas on assessing the particle size alone; it was analyzed that GC tooth mousse, Clinpro tooth cream, and sodium fluoride gel showed comparative results.

Sodium fluoride varnish was found to be the most effective in caries prevention and remineralization which is supported by Edenholm et al.,[4] when compared with other topical application of fluoride. Our results do not agree with Grodzka et al.[5]

Some in vivo studies have reported a significant retention of fluoride from several weeks[6] to 2 year[7] after treatment with the fluoride-containing varnish, whereas Dijkstra et al. (1982),[8] found that all fluoride deposited in and on the enamel after a single in vivo application with varnish was lost within 1 week.

The use of fluoridated solutions has achieved a significant level of popularity among the public, due to the health-care programs, particularly those involving school-age children. Ripa et al.[9] Bruun et al.[10] and Möberg Sköld et al.[11] show the anticaries potential of commercial products containing fluoride solution. In the present study, globular precipitates and amorphous structure of calcium fluoride were observed on enamel surface treated with sodium fluoride solution, and we can assume that it will serve as a reservoir and thus contribute to the caries preventive effect.

When sodium fluoride gel was applied on the sound enamel surface, spherical globular agglomerates of calcium fluoride were formed and seen profusely scattered along the porous defects on the enamel surface. The size of the crystal formed ranged from 0.05 to 0.2 μm which was of very small size as compared to other groups. From the results, it can be assumed that not much of the fluoride reservoir has been formed on the enamel surface which can contribute to the caries prevention for a long period. This result is in favor of different studies which give, mean treatment effect not clinically relevant after the use of sodium fluoride gel, i.e., van Rijkom et al.,[12] Ersin et al.,[13] and Ferreira et al.[14]

The results of the present study showed that among the remineralizing agents used, GC Tooth Mousse Plus was found to be effective and better then Clinpro Tooth Creme. Hegde et al.[15] Patil et al.[16] conducted a comparative evaluation between CPP-ACP and Clinpro tooth cream and gave contrasting results, where they found Clinpro tooth cream to be highly effective with more remineralization properties as compared to CPP-ACP. When Clinpro Tooth Creme and GC Tooth Mousse Plus were compared with other sodium fluoride agents, the fluoride incorporation into the enamel surface was less than the sodium fluoride varnish and solution but were in comparison with that of the sodium fluoride gel. This could be because of the low fluoride concentration present in CPP-ACP (0.2% or 900 ppm of NaF) and Clinpro tooth cream (0.21% or 950 ppm of NaF), initial remineralization was low but long-term use of this product remineralize the enamel surface to the same level as with other fluoride agents.[17]

One of the advantages of these low fluoride remineralizing products is in the treatment of visible white spots lesion where we cannot use highly concentrated fluoride. The synergistic anticariogenic effects are seen when CPP-ACP and beta tricalcium phosphate (β-TCP) are combined with fluoride. The fluoride ions are adsorbed onto the surface of enamel crystals, inhibiting dissolution, and increasing remineralization. The softened surface lesions remineralize faster and more completely than subsurface lesions. With the use of low fluoride concentration as is present in CPP-ACP (0.2% or 900 ppm of NaF) and Clinpro tooth cream (0.21% or 950 ppm of NaF), there is a complex localization of free calcium phosphate and fluoride ion activities, which helps in maintaining a state of supersaturation by suppressing the demineralization. Results show that long-term use of this product will remineralize the enamel surface.
Conclusion

Sodium fluoride varnish is the best fluoride delivery vehicle among the used sodium fluoride agents. CPP-ACP (Tooth Mousse Plus™) and Tri-calcium phosphate with fluoride (Clinpro tooth crème) are excellent delivery vehicles available in a slow release amorphous form to localize fluoride at the tooth surface.

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Conflicts of interest

There are no conflicts of interest.

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