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# Comparison of remineralizing effect of organic and inorganic fluoride by evaluation of microhardness and quantitative analysis of calcium and phosphorus ratio on enamel surface: an *in-vitro* study

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INFORMATION

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ABSTRACT

**Background:** Enamel is a highly mineralized tissue of the body which is composed of 96% inorganic salts and 4% organic matter. Enamel is permeable to water and ions, particularly cations and low molecular weight substances. The enamel continues to mature even after eruption with mineral replacing protein. Recently, the interest on the development of calcium phosphate-based remineralization technology has been increased that led to the development of various remineralizing agents like Fluoride, CPP-ACP (Tooth Mouse plus), Bioglass (Novamin), Ozone, Xylitol, Sensistat etc.

**Aim:** Aim of the present *in vitro* study was to evaluate and compare the remineralizing effect of Organic fluoride (AmF) and inorganic fluoride (NaF) by evaluating Vickers microhardness and quantitative analysis of Calcium and Phosphorus ratio on enamel surface using Scanning Electron Microscope-Energy Dispersive X-ray analysis.

**Materials and methods:** Sixteen maxillary central incisors were decoronated at the cement-enamel junction and mounted in cylindrical moulds filled with self-cure acrylic resin. Artificial demineralized lesions were created on the enamel surface by suspending them in 0.1 M Citric acid buffer at pH of 3.2 for 72 hrs. The samples were then randomly divided into two groups and labelled, Group A – remineralized with NaF for 3 minutes twice daily for one week and Group B - remineralized with AmF for 3 minutes twice daily for one week. Microhardness & SEM-EDX analysis were done before demineralization, after demineralization and after remineralization.

**Results:** Data were analyzed by comparing the mean values between the groups using independent sample t-test. The intra-group analysis was done using repeated-measures ANOVA with posthoc Bonferroni test, and a p-value of <0.05 was considered statistically significant.

**Conclusion:** Organic Fluoride resulted in better remineralization than inorganic Fluoride. After remineralization for one week, enamel samples treated with AmF demonstrated a statistically significant increase in mean microhardness and Ca:P ratio when compared to enamel samples treated with NaF.

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## 1. Introduction

Enamel is a highly mineralized tissue of the body, which is composed of 96% inorganic salts and 4% organic matter [1]. Enamel is permeable to water and ions, particularly cations and low molecular weight substances. The enamel continues to mature even after eruption with mineral replacing protein [2].

For many years, dental caries was considered as a progressive demineralization of enamel apatite followed by degradation of dentin. However, the present concept identifies caries as a dynamic process which can be conceptualized as an imbalance between mineral loss called demineralization and mineral gain called remineralization. Ultimately the net loss of mineral determines the progressive nature of caries.

The various causes of demineralization are acid dissolution of tooth mineral by plaque bacteria, other acidic sources (like carbonated soft drinks, citrus fruit juices, gastric reflux or regurgitation), decreased salivary flow [3] and sometimes intentional demineralization for micromechanical bonding of adhesive restorative materials. If the demineralization phase continues for a longer period, excessive loss of minerals results leading to loss of enamel structure and cavitation – the typical characteristics of dentinal caries.

During the past few years, there has been increased interest and development in calcium phosphate-based remineralization technology [4]. It is enhanced by providing low levels of Calcium and Phosphorus in conjugation with minimal amounts of Fluoride. A variety of remineralizing agents like Fluoride, Casein phosphopeptide- Amorphous calcium phosphate (CPP -ACP) (Tooth Mouse plus), Bioglass (Novamin), Ozone, Xylitol, Sensistatetc, that aid in remineralization of tooth structure are available commercially.

Fluoride is considered as the cornerstone of modern non-invasive dental caries management. Anticaries action of fluoride is due to formation fluorapatite, which is more acid-resistant than hydroxyapatite; enhances remineralization; inhibits ionic bonding during pellicle and plaque formation. In addition, fluoride also has an antibacterial effect [5].

Different types of fluorides used in dentistry are; Sodium fluoride (NaF), Sodium mono-fluorophosphate, Stannous fluoride & Acidulated phosphate fluoride (APF). These are inorganic fluorides and are available as varnishes, solutions, foams, gels, dentifrices etc. [6]. The important factor, which can effectively inhibit the caries is the bioavailability of fluoride. This availability of fluoride depends on its rate of solubility and the capability to adhere to the enamel [7]. In 1957, Muhleman *et al.* reported that organic fluoride (amino fluoride compounds) inhibits caries better than inorganic fluorides [8].

AmF is an organic compound such as N-octa decyl trimethylenediamine-N,N,N-tris(2-ethanol)-dihydro fluoride [$C_{27}H_{58}N_2O_{32}HF$], which consists of two functional groups such as a cationic amino organic group and abounds ionic fluoride group [9]. Recently, AmF containing dentifrices and mouth rinses are commercially available. However, limited research is available on comparison of the remineralization efficacy of various fluorides. Therefore, this study was designed to evaluate and compare the effect of organic fluoride (AmF) and inorganic fluoride (NaF) on calcium and phosphorus ratio on the enamel surface. The null hypothesis for this study was that there would be no difference between organic and inorganic fluoride in remineralization of enamel carious lesion.

## 2. Materials and methods

Sixteen permanent maxillary central incisors extracted for periodontal reasons were included in the study. The teeth were washed thoroughly and polished. The teeth were decoronated at cement-enamel junction using a high-speed diamond disc and mounted in acrylic resin. Subsequently, the mineral content and the microhardness were evaluated. The specimens were subjected to evaluate the mineral content (Ca/P ratio) and microhardness.

### 2.1 SEM EDX analysis

The mineral content (Calcium and Phosphorous) was evaluated using SEM-EDX (Zeiss Evo-18 model SEM, with EDX attachment of the Oxford model) prior to demineralization. The specimens were gold-sputtered and were subjected to scanning electron microscopy at 15.0 kV, and magnification of 10,000 X.

### 2.2 Vickers microhardness testing

The microhardness was evaluated using Vickers hardness tester ((UHL VMHT DIN 50 133). The specimens were placed on the Vickers hardness tester, and a load of 100g was applied for 15 seconds to produce the indentation. The indentations were made at three different points, and the mean of 3 measurements was recorded as baseline microhardness values.

### 2.3 Lesions creation on specimens

Artificial caries like lesions were created on specimens by suspending them in an artificial caries system, which is 0.1M citric acid buffer at pH of 3.2 for 72 hours [10]. After this procedure, the microhardness and mineral content of the specimens were again evaluated.

### 2.4 Treating the specimens with organic and inorganic fluorides

The samples were divided into two study groups (Figure 1) with eight specimens in each group. The experimental groups were distributed as follows:

Group A – Samples were treated with Sodium fluoride (NaF) for 3 min twice daily for one week.

Group B – Samples were treated with Amine fluoride (AmF) for 3 min twice daily for one week.

Specimens were again evaluated for their microhardness and mineral content after remineralization process for one week. In between treatment, the samples were stored in artificial saliva [10].

### 2.5 Statistical analysis

The obtained data were analyzed using statistical package for social sciences, SPSS 21.0, USA. The mean values were compared between the groups using independent sample t-test. The intra-group analysis was done using repeated-measures ANOVA with posthoc Bonferroni test, and a p-value of <0.05 was considered statistically significant.

## 3. Results

### 3.1 SEM-EDX Analysis

The SEM-EDX analysis (figures 2) revealed that after remineralization, calcium content increased significantly in the group treated with AmF when compared to NaF group. In contrast, there was no statistically significant difference in phosphate content in both groups. It also revealed that there was a statistically significant increase in fluoride level in AmF group when compared to NaF group (Table 1). ANOVA analysis showed significant differences in the calcium content ($p=0.009$), Ca:P ratio ($p=0.003$) and fluoride levels ($p=0.001$) after remineralization between the enamel samples treated with NaF and AmF (Table 1).

### 3.2 Vickers microhardness test

The enamel samples treated with AmF (Group B) demonstrated a statistically significant increase in mean microhardness when compared to enamel samples treated with NaF (Group A). Even though NaF group showed an increase in microhardness, but it is lower than baseline value which was statistically significant ($p=0.001$) while AmF group showed an increase in microhardness almost equal to the baseline value (Tables 2). However, no significant differences were observed among the specimens after demineralization.

## 4. Discussion

Fluorides are an important adjunct in the prevention of dental caries. Fluoride ions combine with hydroxyapatite crystals of enamel and forms fluorapatite

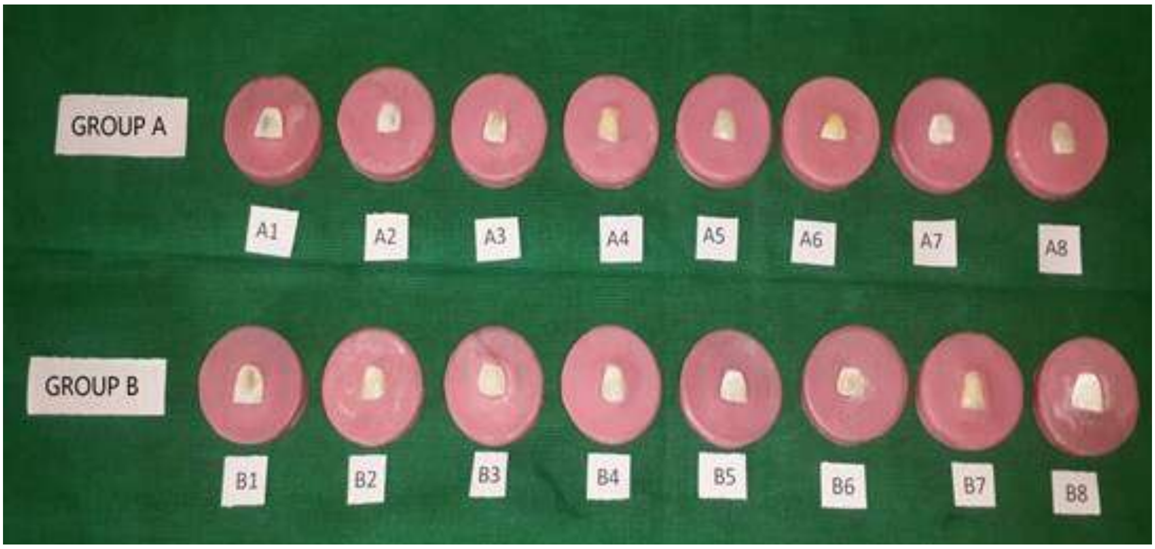


**Figure 1: Decoronated maxillary central incisors mounted on acrylic resin .**

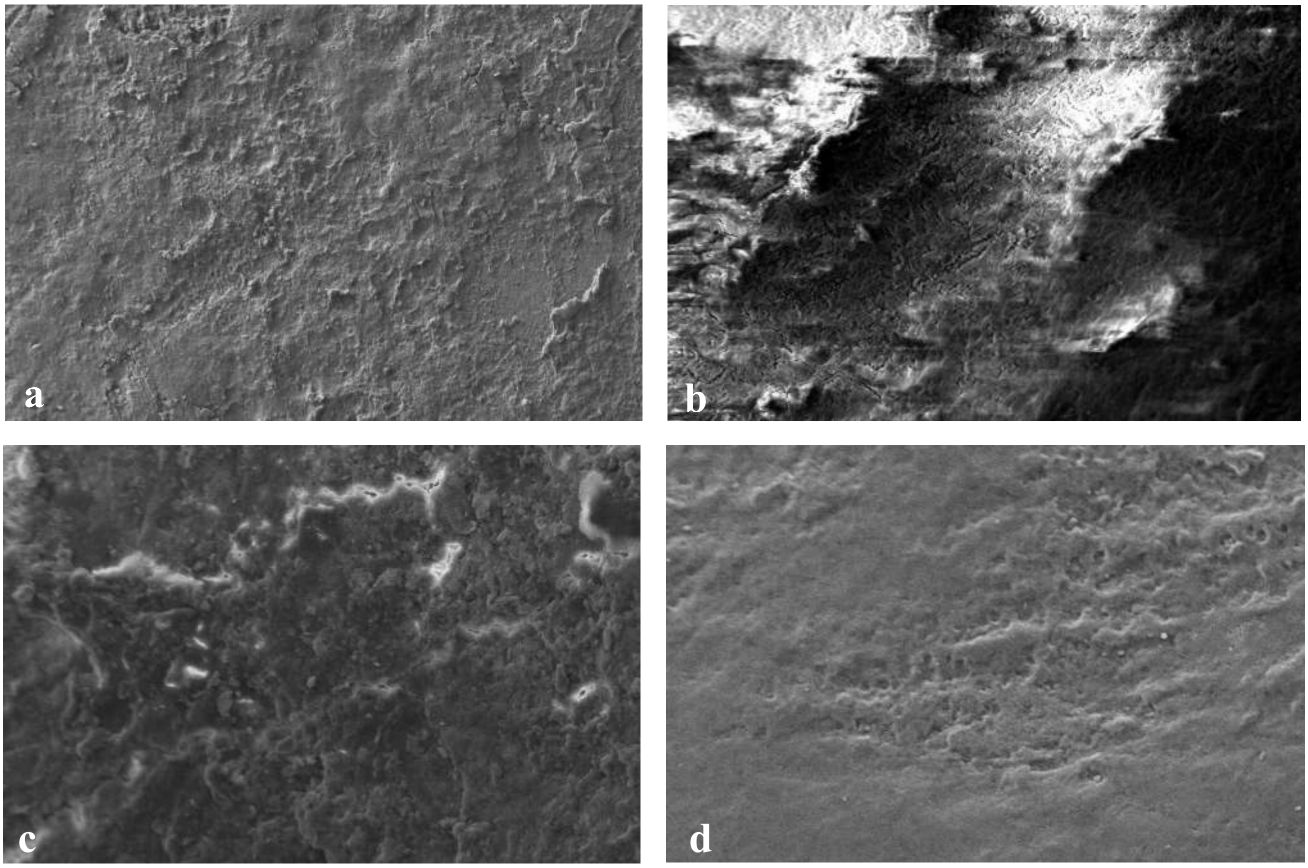


**Figure 2: SEM-EDX analysis, where a. before demineralization, b. after demineralization for 72 hours , c. after remineralization for 1 week with sodium fluoride, and d. after remineralization for 1 week with amine fluoride**

crystals, which enhance the remineralization of enamel [11]. Slow and sustained release of fluoride is necessary to have better deposition on the tooth surface and also to have its effectiveness over longer periods. Calcium ions combine with fluoride and forms the calcium fluoride ($CaF_2$), which results in slow release of fluoride and maintains the salivary fluoride level.

Dentifrices and mouth-rinses are the most commonly used topical agents. Numerous studies suggested that the use of fluoride mouth-rinses as they resulted in higher levels of oral fluoride retention than fluoride dentifrices [12]. Therefore, fluoride mouth-rinses were employed in this study.

Nozari *et al*. [13] reported that citric acid, lactic and acetic acids were all capable of demineralization and reduction of enamel micro hardness. In the present study, a solution of 0.1 M citric acid and 0.1 M Sodium citrate at pH 3.2 was used for demineralization of enamel samples [10]. The demineralization protocol was designed for 72 hours to simulate the duration that occurs in the oral cavity in caries susceptible individuals.

There are different methods such as direct and indirect techniques are available for evaluating demineralization and remineralization of enamel. Direct techniques include longitudinal microradiography, transverse microradiography and wavelength-independent X-ray microradiography. Indirect techniques are polarized light microscopy, microhardness measurement methods, Quantitative energy dispersive X-ray analysis, and iodide permeability.

The average microhardness value for human enamel was reported to be in the range of 270-370 KHN, the value in VHN range from 250-360 VHN which are very similar to each other. Also, the micro hardness (KHN and VHN) values are identical in dentin, where it is reported to be 50-70 KHN or 50-60VHN [14]. In the present study, Vickers hardness (VHN) was preferred over Knoop's hardness (KHN) because the square shape indent obtained in VHN is more accurate to measure.

**Table 1. Inter-group comparison of EDX analysis of Ca, P, Ca: P and F content**

| | | Groups | | | | Significance |
|---|---|---|---|---|---|---|
| | | NaF | | AmF | | |
| | | Mean | SD | Mean | SD | |
| Ca | Baseline | 68.64 | 3.23 | 67.64 | 2.42 | 0.497 |
| | Demineralization | 32.79 | 4.70 | 34.72 | 2.69 | 0.329 |
| | Remineralization | 34.45 | 3.95 | 39.55 | 2.67 | 0.009 |
| P | Baseline | 31.36 | 3.23 | 32.85 | 2.24 | 0.304 |
| | Demineralization | 17.65 | 2.33 | 17.99 | 1.02 | 0.711 |
| | Remineralization | 15.75 | 1.08 | 14.98 | 1.24 | 0.203 |
| Ca:P | Baseline | 2.20 | 0.21 | 2.06 | 0.10 | 0.112 |
| | Demineralization | 1.85 | 0.11 | 1.93 | 0.11 | 0.172 |
| | Remineralization | 2.18 | 0.20 | 2.61 | 0.27 | 0.003* |
| Fluoride | | 1.83 | 0.98 | 5.47 | 1.24 | 0.001* |

* Significant differences were observed among the groups.

**Table 2. Inter-group comparison of Vickers microhardness**

| | | Groups | | | | Significance |
|---|---|---|---|---|---|---|
| | | NaF | | AmF | | |
| | | Mean | SD | Mean | SD | |
| VHN | Baseline | 380.88 | 23.65 | 387.88 | 25.26 | 0.576; NS |
| | Demineralization | 302.25 | 11.25 | 307.75 | 21.90 | 0.538; NS |
| | Remineralization | 342.13 | 21.38 | 385.25 | 20.57 | 0.001; Sig |

* Significant differences were observed between the groups.

The baseline microhardness values obtained in the present study were in the range of 380.87-387.87 VHN. But, a decrease in the surface microhardness values for both the groups (302.25 and 307.75 VHN respectively) was observed after the demineralization process for 72 hours. After remineralization, the mean microhardness in Group A (NaF) increased to 342.12 VHN, whereas in Group B (AmF) it was 385.25 VHN (Table 2).

The results of the present study were in agreement with an investigation by Priyadarshini *et al.* [15]. They also suggested that AmF compounds result in a marked increase in enamel microhardness when compared to NaF. On the contrary, Lippert *et al.* [16] compared the anticaries potential of two new commercial dentifrices, which contain AmF and NaF; by measuring Vickers hardness and concluded that NaF showed superior anticaries potential when compared to AmF.

The EDX analysis of this study revealed that there was a statistically significant decrease in calcium and phosphate levels in both the groups after demineralization (figure 2.b). After remineralization, calcium content increased significantly in the group treated with AmF (figure 2.d) when compared to NaF group (figure 2.c).

In contrast, there was no statistically significant difference in phosphate content in both groups. It also revealed that there was a significant increase in fluoride level in AmF group when compared to NaF group (Table 1).

When enamel surface is treated with any fluoride dentifrice, the following reaction is anticipated.

$$Ca_{10}(PO_4)_6(OH)_2 + 20F^- \rightarrow 1OCaF_2 + 6P0_4 + 20H^-$$

$$\downarrow$$

$$Ca_{10}(PO_4)_6(F)_2$$

It is observed that calcium in hydroxyapatite remains in the salt as CaF2 while phosphorous content is released into the liquid as phosphate ions [17]. This reaction further progresses to form fluorapatite. This study revealed that Ca:P ratio increased in both the groups but the amount of increase is statistically more significant in the AmF group when compared to NaF group. This may be due to the advantageous characteristics of AmF, including its ability as a surface-active agent, which has tensioactive and anti-glycolytic properties. The surface-active property provides self-alignment of the hydrophilic part towards the tooth surface and the hydrophobic part towards the oral cavity that result in an accumulation of fluoride very close to the tooth surface. This accumulated fluoride readily combines with calcium and forms calcium fluoride, which acts as a fluoride reservoir [18].

The superior anti-cariogenic property of AmF can be explained by two reasons such as (a) Presence of fluoride, (b) the antiplaque effect of amine (organic) component that has the inhibiting effect on bacterial adhesion. Therefore, AmF allows accumulation of fluoride close to the tooth surface, providing a sustained fluoride release. Various studies have reported the AmF's anti-caries effects [19] based on their surface-active/tensioactive property that is leading to the fast distribution of fluoride and homogenous coating on the tooth surface for a prolonged period.

In the present study, treatment with NaF showed less remineralization on enamel surface. The reason for the less remineralization can be attributed to the formation of thick calcium fluoride layer on the tooth surface that might result due to the reaction between NaF (inorganic fluoride) and hydroxyapatite of enamel. This thick calcium fluoride layer inhibits further diffusion of fluoride from the topical fluoride agents, thus providing a relatively lower bioavailability of fluoride ions [20]. Further, the sodium cations do not have self-governing caries prophylactic property.

Arnold *et al.* [21] using polarized light microscopy had reported that the more stable superficial enamel layer was formed after treating with AmF compared to the treatments done with NaF or sodium monofluorides. In a study, Sefton J *et al.* [22] also suggested that the more amount of fluoride was deposited on enamel by treating it with AmF than sodium or stannous fluoride. Another study by Naumova *et al.* [23] used different amine concentrations on enamel remineralization. They concluded that the thickness of the superficial layer increased with decreasing fluoride concentrations, whereas Ca and P content increased with increasing fluoride concentration.

The results of this study suggested that use of NaF and AmF remineralizing agents results in remineralization of incipient lesions, thereby preventing further destruction of the tooth. Among the study groups, Group B (AmF) showed a more significant effect in remineralization when compared to Group A (NaF). Therefore, the null hypothesis for this study that organic and inorganic fluorides have a similar impact on remineralization of carious enamel lesion has been rejected.

## 5. Conclusion

Within the limitations of this present in vitro study, the following conclusions were drawn;

- Both inorganic (NaF) and organic fluorides (AmF) were effective in remineralization. However, the enamel samples treated with AmF showed more Ca:P ratio compared to the treatment with NaF.
- Also, compared to NaF remineralization, AmF remineralization demonstrated a significant increase in mean microhardness, which is equivalent to the baseline values.

Further *in vivo* studies are to be undertaken to evaluate the efficacy of these remineralizing agents in remineralizing incipient lesions.

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