

## Evaluation of Ability of Dentifrices to Remineralize Artificial Caries-Like Lesions

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### ABSTRACT

**Background:** Human endeavors to prevent dental caries have led to the use of different modalities and agents, the most commonly used of which is fluoridated dentifrice. An *in situ* study was carried out to evaluate the efficacy of fluoridated dentifrices in achieving remineralization of initial caries-like lesions using surface microhardness measurements and to study the qualitative changes by scanning electron microscopy.

**Methods:** Sixteen children 12-16 years of age wore a specially fabricated appliance with an artificially demineralized enamel slab for 24 hours a day, for four weeks. The children were divided into two groups, A and B. Following one week use of placebo dentifrice by both groups, group A used a fluoride dentifrice containing 1000 ppm sodium monofluorophosphate, whereas group B used a placebo twice daily for 5 minutes for 21 days. Surface microhardness test carried out using a Knoop diamond indenter followed by scanning electron microscopy to evaluate the lesions. The results were statistically analyzed using the student t test. P value less than 0.05 was considered significant.

**Results:** The average hardness recovery for the experimental group was significantly higher than that in the control group ( $P < 0.001$ ). Scanning electron microscopy revealed that fluoride significantly enhances remineralization of initial caries-like lesions.

**Conclusion:** Regular use of fluoridated dentifrices significantly enhances remineralization of white spot lesions.

**Keywords:** Dental caries, Enamel microhardness, Fluoridated dentifrices, Tooth remineralization.

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### Introduction

Considerable evidence exists to suggest that developed countries have experienced a decline in dental caries prevalence in the recent decades through extensive use of fluoride (F) but, the incidence of dental caries in developing countries is still high.<sup>1-4</sup> One way in which caries control may be achieved in developing nations is through regular use of fluoride containing dentifrice combining the mechanical effect of tooth brushing with delivery of fluoride to the plaque tooth interface. Extensive experimental, clinical and epidemiological researches carried out to explore the multifaceted nature of the preventive role of fluorides that proved the effectiveness of fluoridated dentifrices as an integral

part of caries preventive strategies.<sup>5-9</sup> Artificial caries-like lesions can be produced in enamel *in-vitro* to show all the principal histological features of natural caries; and remineralization of these artificial carious lesions can be demonstrated using scanning electron microscopy.<sup>10</sup> Artificial caries-like lesions of enamel are more homogeneously reproducible than natural lesions and an area of enamel having a defined lesion of constant depth beneath the surface can be produced to allow different areas of the same lesion to be used for testing remineralization phenomenon. A number of *in situ* remineralization studies previously conducted showed that properly formulated sodium fluoride

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dentifrices provided partial remineralization of artificially-formed caries lesions within a two-week treatment period.<sup>11</sup> The objective of this study was to evaluate the efficacy of fluoridated dentifrices in remineralization of initial caries-like lesions using surface microhardness measurements and to study the qualitative changes by scanning electron microscopy.

## Materials and Methods

### *Subjects and experimental procedures*

Sixteen healthy children aged 12-16 years voluntarily participated in the study which was approved by the Ethics Committee. The participants were residents of a residential school and had similar dietary habit and life style. All children had no clinically detectable carious lesions or history of illness in the recent past period, and were regular in attending the school. The children had a good periodontal health, were not undergoing any orthodontic treatment and had no systemic or topical exposure to fluoride in the recent past period. The study was described for them, and they were instructed not to use any oral hygiene products other than those assigned to them during the study. A written consent was obtained from the concerned school authorities and parents prior to the commencement of the study.

### *Preparation of decalcified enamel slabs*

Enamel samples were prepared by cutting 4-mm-diameter cores from 34 human premolars extracted for orthodontic purpose using a diamond core drill. The teeth, collected from local dentists, were stored until use in a 1.8% formaldehyde solution, maintained above pH 7.5. The enamel cores were mounted in ¼ inch-diameter lucite rods with a dental acrylic covering all sides except the surface. At least 40 µm of the enamel surface were removed by polishing with 600 grit silicon carbide water slurry. The surface was then polished to a mirror finish using gamma alumina. Any enamel cores found to have surface imperfections or white spots were rejected. The enamel cores were tested for microhardness values before decalcification. The enamel was decalcified by suspending each lucite rod in a demineralizing solution for 48 hours at 37°C, after which the solution was changed and the slabs were kept for another 3 days. The solution

consisted of 3.1 mmol/L calcium chloride, 3.1 mmol/L sodium dihydrogen orthophosphate and 50 mmol/L glacial acetic acid. pH of the solution was adjusted to 4.5 using 1 mol/L sodium hydroxide. This method produced artificial lesions similar to natural white spots.<sup>10</sup> Lesion depths were approximately 120 µm. The enamel cores were removed from the lucite rod and cut to a 3-mm thickness to give a 4 × 4 × 3 mm slab. The enamel slabs thus produced and were incorporated in the left and right buccal flanges of specially fabricated appliances. The appliance design was a modification of the original intraoral cariogenicity test appliance designed by Koulourides.<sup>12</sup> The appliances were designed to hold two enamel slabs in different proximal positions adjacent to natural teeth (Figure 1). The dentifrices used for this investigation were: (1) dentifrice containing 1000 ppm sodium monofluorophosphate and a fluoride-compatible silica abrasive system (2) a non-fluoride-silica abrasive placebo. Dentifrices were formulated to be similar in color and flavor and were packed in 5-oz. white tubes.



**Figure 1.** It shows a patient wearing the intraoral appliance with the enamel slab

### *Protocol*

Dentifrice treatment outcomes were compared using a randomized block test design with 16 volunteer panelists. A four week treatment regimen was employed to assess the remineralization resulting from each dentifrice use. At the beginning of each four week test, panelists were given instructions on proper brushing technique and maintenance of the

appliance. For the first week of each four-week test, children used a placebo dentifrice (labeled "Use First") exclusively. This was to allow a salivary film to form on the enamel slabs for a full week prior to exposure to high concentrations of fluoride. Such acquired films are similar, histochemically, to the natural pellicle films observed on extracted teeth. During the following three weeks, dentifrice (labeled "Use Second") was used by the children. Half of the children received the test dentifrice. These children formed Group A and the other half used the placebo dentifrice formed Group B. During the study period, all children wore the appliances for 24 hours even while sleeping and eating. The appliances were only removed for short periods of cleansing. The participants used no other oral hygiene aid except for the prescribed dentifrice. At the end of the study period, the appliances were removed from the mouth and washed with de-ionized water. The enamel slabs were removed and washed with de-ionized water after which they were stored in moist conditions in labeled containers till analysis. Surface microhardness test was carried out to assess the degree of lesion demineralization and remineralization using a Knoop diamond indenter at a 200 g load at X200 magnification. Scanning electron microscope was used to observe the surface topography of the enamel slabs at magnifications of 1K (X1000), 2K (X2000), 5K (X5000) and 10K (X10000) after demineralization and after remineralization. The enamel samples were coated with 20  $\mu\text{m}$  thick film of Gold-Palladium which is a highly efficient con-

ductor of electrons and therefore, gives clearer pictures.

### Analysis of data

The data obtained were subjected to statistical analysis using the student t test.

### Results

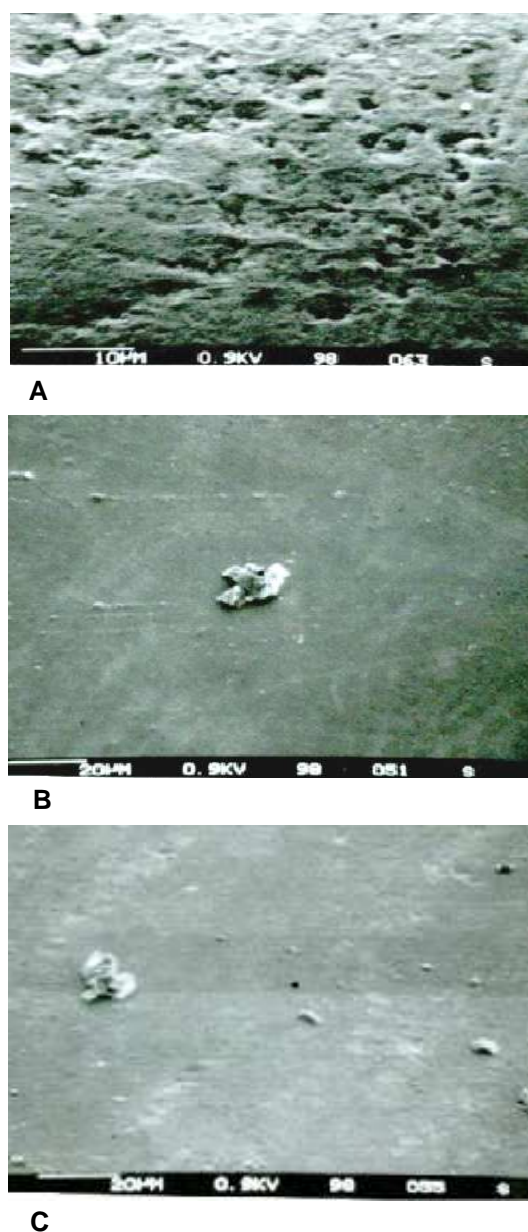
The mean  $\pm$  SD surface microhardness value of the untreated, polished enamel slabs was  $316.18 \pm 9.53$  KHN. After in vitro demineralization, the mean value changed to  $124.43 \pm 37.85$  KHN ( $P = 0.00$ ). Table 1 shows the mean microhardness values of group A and B at the initial stage, after demineralization and after 3 weeks of intraoral exposure and also the comparison of changes between two groups.

On observation under a scanning electron microscope, demineralized enamel slabs depicted features of demineralized zones similar to caries-like lesions. At 1000 magnification, deep pits in honeycomb pattern were observed (Figure 2A). After remineralization, the photomicrographs of group A revealed a more regular, smooth and homogenous surface. Discrete areas of crystallite formation were revealed (Figure 2B). Group B slabs revealed surface roughening however, some discrete zones of crystallite formation were observed (Figure 2C). The photomicrographs at 2000 magnification of the demineralized enamel slabs depicted microcavities which were created artificially and situated at different levels in the enamel. At 5000 magnification, numerous depressions in a honeycomb pattern were revealed.

**Table 1.** Comparison of microhardness changes between the two groups.

Stage	Group A (n = 8) Mean $\pm$ SD (KHN)	Group B (n = 8) Mean $\pm$ SD (KHN)
Initial	312.0 $\pm$ 5.66	320.88 $\pm$ 10.62
Demineralization	125.63 $\pm$ 38.19	123.25 $\pm$ 40.10
Remineralization	243.63 $\pm$ 32.3	191.88 $\pm$ 41.41

Percent of recovery was 64.63% and 35.25% in groups A and B, respectively. P value was highly significant ( $P < 0.001$ ).



**Figure 2.** Photograph depicting demineralization and remineralization of enamel slabs (A) Demineralized enamel. (B) After fluoride dentifrice use. (C) After placebo dentifrice use.

## Discussion

The mean of hardness values of normal enamel slabs was  $316.18 \pm 9.35$  KHN. Also, the average microhardness of demineralized enamel slabs was  $124 \pm 37.8$  KHN, which was in agreement with the values reported previously by other investigators, who reported the KHN microhardness of demineralized enamel to range from 119 to 154.<sup>13</sup> Other authors stated that the KHN for white spot lesions ranges from 100 to 250.<sup>14</sup> The enamel slabs of the

test group, exhibited an average microhardness of  $243.63 \pm 32.30$ . The average percentage recovery in hardness was 64.6%, whereas in the control group an average microhardness of  $191.88 \pm 41.41$  was observed. The average hardness recovery in percentage for this group was 35.25%. Though increase in surface hardness was observed in both groups, it was comparatively more in fluoride dentifrice group, and was statistically significant.

The demineralized enamel slab was observed at different magnifications. The depicted microcavities situated at different levels in the demineralized enamel at 2000 magnification, were similar to those observed by Thylstrup.<sup>15</sup> At 5000 magnification, numerous depressions in a honeycomb pattern were revealed which corresponded to the observations made by other investigators.<sup>16,17</sup>

The cariostatic effects of dentifrices are ascribed to their ability to increase the rate of repair of carious enamel lesions through remineralization and increasing the resistance of remineralized areas to secondary acid attack.<sup>18-22</sup> Demineralization of the teeth was carried out as described by Stephen, et al.<sup>23</sup> using a buffer solution system which created lesions over a period of 5 days at 37°C. Presently, the most extensively used fluoride delivery system is fluoride dentifrice. Fluoride concentration of dentifrices ranges between 850-1200 ppm, hence dentifrices containing 1000 ppm fluoride were used in the study. Sodium monofluorophosphate is known to be a superior cariostatic agent as it maintains the apatite structure of enamel. It is also less susceptible to inactivation by dentifrice abrasive systems.<sup>24</sup> Caries inhibition has been attributed either to the specific monofluorophosphate anion or to the fluoride arising from monofluorophosphate in the oral environment due to hydrolysis. Some authors report that phosphates and fluorides act independently in reducing caries.<sup>25</sup> Other beneficial properties of this salt described by White,<sup>26</sup> make it more appropriate for this application. The present concepts of the cariostatic mechanism of action of topical fluoride application developed during the last few years, established that the calcium fluoride particles that form on dental enamel on application of high concentrations of fluoride are not lost during the subsequent 24 hours, as previously thought, but are retained for extensive periods of time and provide free fluoride during pH cycles in the dental plaque.<sup>27,28</sup> Calcium fluoride thus constitutes a reservoir of fluo-

ride that releases fluoride when the pH drops to 6 or below in dental plaque. This is caused by surface adsorption of secondary phosphate onto the calcium fluoride crystals. This process inhibits the dissolution of this mineral at neutral pH. When plaque pH drops, secondary phosphate is converted to primary phosphate which is not able to inhibit the dissolution of calcium fluoride on the enamel surface. Calcium fluoride thus provides a pH controlled reservoir of fluoride, which is mobilized on a carious challenge and helps in remineralization reactions in the enamel.<sup>29</sup> Fluoride-containing toothpastes that are applied 1-2 times daily can secure the deposition of reservoirs of calcium fluoride on the enamel, underneath the plaque.

However, fluoride has shown limitations in the intra-oral experiments. When the pH drops significantly under thick plaque, even high concentrations of fluoride cannot inhibit caries completely.<sup>30</sup> Such experiments offer a possible explanation for the clinical observation that 10-15% of patients exhibit caries even after fluoride application. This could be explained by the fact that if the oral hygiene is improper, thick plaque forms on the enamel and the pH drops so low that even fluorapatite is unstable. Improved oral hygiene and the use of fluoride may thus be beneficial; the two procedures have a synergistic effect. Hence, sodium monofluorophosphate dentifrice was used in the present study to evaluate its cariostatic properties in the oral environment using an in-situ model. The present findings are in agreement with the previous in vitro as well as in vivo studies confirming remineralization of artificial carious lesions<sup>31,32</sup> reported that in vivo remineralization resulted in a more regular, smooth and homogenous surface. It can be concluded that the use of fluoride dentifrice results in a more homogenous surface with densely packed crystallites. Similar findings were reported previously.<sup>32</sup> Under clinical conditions, it can be questioned if this is a result of remineralization alone or a combination of remineralization and mechanical wear through brushing. The appearance of the surface after treatment supports this assumption. Artificially produced caries-like lesions are of considerable interest as they can be compared to the earliest detectable ultrastructural change in the caries process. The observed plugging of the pores and interprismatic areas are due to precipitation of insoluble crystallite material of

unknown origin (probably calcium fluoride or fluorapatite). This 'plugging' by fluoride ions could influence the transport of ions within the lesion and prevent further diffusion of ions out of the surface layer. Thus, the clogging of pores could prevent further dissolution and subsequent demineralization of enamel.

## Conclusion

The results suggest that fluoridated dentifrice enhances remineralization of initial caries-like lesions.

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