Remineralizing Effectiveness of Calcium Sucrose Phosphate and Fluoride Dentifrices: An *In vitro* Study

Abstract

Context: Dentifrices-containing remineralizing agents are known to be effective in remineralization of early enamel lesions. **Aims:** This study aimed to compare and evaluate the changes in surface roughness, surface morphology, and mineral content of demineralized enamel lesion after treatment with dentifrices-containing sodium monofluorophosphate, amine fluoride, and Anticay® (calcium sucrose phosphate with inorganic amorphous calcium phosphate). **Settings and Design:** This was an *in vitro* study. **Subjects and Methods:** Eighteen extracted maxillary molars were decoronated and sectioned into four to obtain 72 specimens. Specimens were demineralized and randomly divided into four different test groups: Group A: no treatment (control), Group B: sodium monofluorophosphate dentifrice (Colgate), Group C: amine fluoride dentifrice (Amflor), Group D: Anticay® dentifrice (EnaFix) and subjected to pH-cycling for 4 weeks. After 4 weeks, they were assessed using a profi lometer and scanning electron microscope (SEM)-energy dispersive X-ray analysis (EDAX) for changes in surface roughness, surface morphology, and mineral content. **Statistical Analysis Used:** Intergroup comparison was done using repeated measures ANOVA. **Results:** Intergroup comparison revealed no significant difference in surface roughness and mineral content after remineralization between the groups. SEM images showed mineral deposition in all the dentifrice groups obliterating the defects caused due to demineralization. **Conclusions:** Sodium monofluorophosphate, amine fluoride-containing dentifrices, and calcium sucrose phosphate with inorganic amorphous calcium phosphate-containing dentifrice were found equivocal in their remineralizing effectiveness of early enamel lesions.

Keywords: *Calcium sucrose phosphate, dentifrice, fluoride, remineralization*

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Introduction

The focus in caries management has shifted in recent years to noninvasive treatment of early enamel lesions by means of remineralizing agents. Caries results from an imbalance between many cycles of demineralization and remineralization rather than from continued demineralization. The earliest clinical sign is the "white spot lesion." Therapeutic intervention if done at this stage can totally arrest or reverse the whole caries process by remineralization.[1]

Fluoride is the most commonly used agent to promote remineralization. This is attributed to the formation of fluorhydroxyapatite which is less soluble under low pH.[2] Dentifrices containing various fluoride compounds such as sodium fluoride, sodium monofluorophosphate, stannous fluoride, and amine fluoride are known to be effective in the remineralization of early white spot lesions.[3-5] Sodium monofluorophosphate is most widely used in dentifrices due to its greater compatibility with dentifrice abrasives.[6] Earlier studies have shown that amine fluoride, a type of organic fluoride-containing dentifrices, is superior to other fluoride dentifrices.[7-10] Dentifrices containing 1000 ppm of fluoride have been recommended for all age groups due to its effectiveness in reducing caries. However, the use of greater amount may increase the risk for fluorosis, especially among children <6 years of age.[11] Hence, nonfluoride-containing dentifrices with remineralizing agents have been developed. Since the process of remineralization is limited by the availability of calcium and phosphorous ions, calcium phosphate-based remineralization systems like Anticay® which is a mixture of calcium sucrose phosphate with inorganic amorphous calcium phosphate are commercially available.[12,13]

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The remineralizing effectiveness of Anticay® in comparison with fluoride dentifrices is not known. Since remineralization results in a change in the surface roughness, morphology, and the mineral content of enamel, these parameters can be used to determine the extent of remineralization. Hence, the present study was carried out to compare the remineralization effectiveness of sodium monofluorophosphate, amine fluoride, and calcium sucrose phosphate with inorganic amorphous calcium phosphate (Anticay®)-containing dentifrices by evaluating the changes in surface roughness, morphology, and mineral content of the demineralized enamel lesion after treatment with dentifrices. The null hypothesis was that there is no difference in the dentifrice groups in terms of surface roughness and mineral content of enamel after remineralization.

Subjects and Methods

The study followed an *in vitro* experimental design and was carried out after obtaining Institutional Ethics Committee clearance. Sample size for assessing change in surface roughness was calculated as 12 in each group, based on the formula E = N-B-T where N is the assumed sample size for each group minus 1; B is the blocking component representing the environmental effects allowed for the design minus 1; and T is the treatment component minus 1 (E=[12-1]-[0-1]-[1-1] =12) and E is the degree of freedom of the error component (desired value between 10 and 20). The sample size for scanning electron microscope and energy dispersive X-ray analysis (SEM-EDAX) was calculated as 6 in each group; keeping the power of the test as 0.25 with a predetermined type 1 error 0.8 as per cumulative distribution function.[14]

Inclusion criteria were permanent maxillary molars with no carious lesions. Teeth with enamel or dentinal defects, erosions, microcracks, visible stains, developmental anomalies, restorations, and sealants were excluded. Eighteen human maxillary molars extracted for periodontal reasons were collected and stored in formalin. The surface debris and calculus were removed using an ultrasonic scaler (Dental EMS Airflow S2 ultrasonic, Nyon, Switzerland) and immersed in distilled water. The teeth were examined under a stereomicroscope (Reichert Star Stereo Zoom, New York, USA) for any defects such as stains or cracks and such specimens were discarded. All the molar teeth were decoronated. The coronal part of each tooth was then sectioned buccolingually and mesiodistally into four sections using a high-speed diamond disc (LM Abrasivi, L. M. Pianotti S. r. l. Grugliasco, Italy) with a slow speed handpiece (Saeyang Microtech, Daegu, Korea) and air-water spray, providing a total of 72 specimens. The four sections of each tooth were identified by a number given to the corresponding tooth. They were randomly assigned to the four test groups, using block randomization method. This ensured that each tooth serves as its own control. Twenty-four sections (six in each group) were used for scanning electron microscope analysis and 48 sections (12 in each group) were used for profilometric analysis.

The enamel surface of the specimens was smoothened with an acrylic trimmer, polished with 300, 600, 1200 grit-sized sandpaper and finished with pumice slurry to obtain a flat surface. The specimens were coated with colored nail varnish leaving a window of approximately 4 mm × 2 mm size at the middle third and then subjected to demineralization. One half of this window (2 mm × 2 mm) was coated with colored nail varnish after demineralization to obtain baseline values. Dentifrice application was done in the exposed 2 mm × 2 mm window. The specimens were put in cassettes and kept immersed in a 300 ml demineralizing solution (pH 4.5) for 96 h and agitated intermittently to induce white spot caries-like lesions. The demineralizing solution contained 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, and 0.05M acetic acid, 1M KOH.[15] The demineralization was confirmed under a stereomicroscope (Reichert Star Stereo Zoom, New York, USA). The various test groups were:

- Group A: No treatment (negative control)
- Group B: Sodium monofluorophosphate dentifrice (Colgate Toothpaste Great Regular Flavor, Colgate, Palmolive Company, Midtown Manhattan, New York) containing 1000 ppm fluoride
- Group C: Amine fluoride dentifrice (Amflor, Group Pharmaceuticals, Goregaon West, Mumbai, India) containing 1000 ppm fluoride
- Group D: 5% calcium sucrose phosphate with inorganic amorphous calcium phosphate (Anticay®) dentifrice (EnaFix, Group Pharmaceuticals, Goregaon West, Mumbai, India)

The specimens in Groups B, C, and D were brushed with the respective dentifrices twice daily for 2 min at 12 h intervals using a powered toothbrush (Colgate Kids Spiderman Battery Operated Toothbrush, Colgate-Palmolive Company, New York, USA) to standardize the force applied during brushing. A smear of toothpaste as recommended by American Academy of Paediatric Dentistry for children under 3 years of age was used.[16] After brushing, the samples were placed in artificial saliva. All specimens, including that of the control group, were subjected to pH cycling (alternative phases of demineralization and remineralization) for a period of 4 weeks. The specimens were brushed twice a day and subjected to 3 h of demineralization twice daily, with 2 h of remineralization between the periods of demineralization.[4] The teeth were placed in 50 ml artificial saliva for the rest of the day.[17] The composition of artificial saliva was 2.2 g/L Gastric mucin, 0.381g/L NaCl, 0.213g/L $CaCl_2.2H_2O$, 0.738 g/L $K_2HPO_4.3H_2O$, and 1.114 g/L KCl with 85% lactic acid and

with pH 7 at 37°C.[18] The remineralizing solution contained 1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, and 0.15M KCl with a pH of 7.[15] Before each cycle, the solutions were freshly prepared and the pH was measured. Separate containers were used for each group, throughout the experiment. At the end of 4 weeks, the nail varnish was peeled off from all of the specimens using cotton swabs soaked in acetone solution (1:1).

The change in surface roughness was measured using a Profilometer (Surface Roughness Tester, SJ-301, Mitutoyo, Kanagawa, Japan) with a 5-μm radius diamond stylus tip under 4-mN load. The stylus traveled across each window of every specimen at a velocity of 0.25 mm/s, perpendicular to the specimen surface. Three traverses of the stylus were randomly made across each window of the specimen. A sampling length of 0.25–0.8 mm was measured. A graph representing the surface profile was obtained. The average surface roughness (R_a) was the arithmetic average height of roughness-component irregularities (as represented in the graph) from the mean line measured within the sampling length (in μm). The profilometer was calibrated before each measuring session.

The samples were dried in a hot air oven before SEM analysis to ensure that the samples were moisture free, and care was taken to avoid any direct contact with air or moisture. The samples were covered with a carbon paper and placed on a metal mounting block, then sputtered with gold in a vacuum-closed container at high temperature. The samples were analyzed using SEM (Jeol JSM-638 OLA, Peabody, Massachusetts, USA) for surface changes. The mineral changes were assessed by EDAX carried out using an energy dispersion X-ray spectrophotometer (Jeol JSM-638 OLA, Peabody, Massachusetts, USA) at ×2000 magnification and 20 kV voltage. The calcium, phosphate, and the fluoride content in enamel after demineralization and after 4 weeks of remineralization were quantified as atomic weight percentage. All readings were recorded by an expert examiner who was not aware of group allocation and not involved in the specimen preparation and intervention methods used in the study.

All data were analyzed using the SPSS version 16 (SPSS, Chicago, IL, USA). Values obtained after demineralization and remineralization were entered, and descriptive statistics was tabulated. Repeated measures ANOVA were performed to check the statistical difference between the groups. Paired *t*-test was performed to analyze the change in the surface roughness and the mineral content after 4 weeks of remineralization within the groups. $P \leq 0.05$ was considered statistically significant.

Results

There was no significant difference in surface roughness and mineral content between the groups after demineralization [Tables 1 and 2].

Table 1: Intergroup comparison of mean average surface roughness (μm) after demineralization and at 4 weeks remineralization

	After demineralization		After remineralization (4 weeks)	
	Mean±SD	CI	Mean±SD	CI
Group A	2.88±1.15	3.61-2.15	2.56±1.12	3.27-1.85
Group B	2.38±0.86	2.93-1.84	1.41±0.55	1.76-1.05
Group C	2.67±0.96	3.28-2.06	1.40±0.58	1.77-1.03
Group D	2.99±1.33	3.83-2.14	1.83±0.87	2.38-1.27

df=3; *F*=2.338; *P*=0.087. SD: Standard deviation; CI: Confidence interval

The maximum decrease in surface roughness (R_a) was seen in the amine fluoride group. Repeated measures ANOVA analysis for intergroup comparison showed that the difference in R_a was not statistically significant between the groups at 4 weeks of remineralization [Table 1]. However, in all the groups, paired t-test revealed a significant decrease in R_a at 4 weeks of remineralization when compared to the values after demineralization [Table 3].

Repeated measures ANOVA analysis for intergroup comparison revealed no significant difference in mineral content post-remineralization between the groups. The calcium, phosphorous, and fluoride content was found to have increased post-remineralization in all the groups. The calcium-phosphorous ratio (Ca/P) was found to have increased in all the groups except control group. The maximum increase in Ca/P was noted in calcium sucrose phosphate group and maximum increase in fluoride content was in sodium monofluorophosphate group [Table 2].

Paired *t*-test [Table 3] revealed a significant increase in both calcium and phosphorus content in the sodium monofluorophosphate group and a significant increase in the fluoride content in the amine fluoride group after remineralization. No significant change in the mineral content was observed in the calcium sucrose phosphate group after remineralization. Significant changes in the phosphorus and fluoride content after remineralization were also noted in the control group.

SEM images' interpretation revealed the following:

Normal enamel shows a characteristic fish scale appearance with a smooth and intact surface.[19] Following demineralization, the rods appeared collapsed due to lack of proper orientation of the hydroxyapatite crystals and the fish scale appearance of normal enamel disappeared. The enamel surface appeared rough and uneven, and increased porosities were observed. A minor honeycomb pattern of demineralized enamel was observed [Figure 1]. After 4 weeks of remineralization, multiple porosities and an irregular surface with slight surface precipitation of minerals were visible in the control group [Figure 2]. All the test groups [Figures 3-5] post-remineralization revealed a layer of surface deposition of minerals

Table 2: Intergroup comparison of mean mineral content after demineralization and at 4 weeks remineralization after 4 weeks

	Group	After demineralization (weight percentage)		After remineralization (4 weeks) (weight percentage)		*F* (df)	*P*
		Mean±SD	CI	Mean±SD	CI		
Calcium	Group A	32.88±2.29	35.28-0.47	33.11±2.11	35.33-0.89	2.481 (3)	0.091
	Group B	33.16±4.39	37.76-8.55	37.67±4.70	42.60-32.73		
	Group C	30.86±2.58	33.56-8.15	31.40±2.18	33.69-9.11		
	Group D	27.19±8.56	36.21-8.16	34.94±4.20	39.34-0.53		
Phosphorus	Group A	19.96±6.20	26.47-3.45	20.22±6.25	26.77-3.66	0.573 (3)	0.639
	Group B	18.49±1.85	20.43-6.55	20.29±1.54	21.90-8.67		
	Group C	19.48±1.51	21.07-17.90	19.32±1.96	21.38-17.25		
	Group D	16.17±2.86	19.17-13.17	19.09±1.41	20.56-17.61		
Fluoride	Group A	0.34±0.31	0.67-0.01	0.33±0.60	0.95-0.26	1.515 (3)	0.241
	Group B	0.66±0.26	0.93-0.38	4.89±8.17	13.46-3.68		
	Group C	0.83±0.50	1.36-0.31	1.34±2.77	4.18-1.36		
	Group D	0.82±0.53	1.38-0.27	0.59±0.82	1.44-0.20		
Ca/P	Group A	1.76±0.46	2.24-1.28	1.75±0.49	2.26-1.24	0.618 (3)	0.611
	Group B	1.80±0.28	2.10-1.50	1.85±0.13	1.99-1.71		
	Group C	1.59±0.15	1.75-1.42	1.63±0.16	1.80-1.46		
	Group D	1.63±0.37	2.03-1.24	1.82±0.13	1.96-1.68		

SD: Standard deviation; CI: Confidence interval; Ca/P: Calcium-phosphorous ratio

Table 3: Comparison of surface roughness and mineral content within groups after demineralization and at 4 weeks remineralization

Groups	Variable	*t*	*P*	CI
Group A	Calcium	1.566	0.178	0.62-0.15
	Phosphorus	3.583	0.016*	0.44-0.07
	Fluoride	2.712	0.042*	0.51-0.01
	Ca/P	0.245	0.816	0.38-0.05
	Surface roughness	3.876	0.003*	0.50-0.14
Group B	Calcium	3.290	0.022*	8.03-0.98
	Phosphorus	2.837	0.036*	3.42-0.17
	Fluoride	1.246	0.268	12.96-4.50
	Ca/P	0.520	0.625	0.28-0.18
	Surface roughness	5.126	<0.001*	1.40-0.56
Group C	Calcium	2.107	0.089	1.19-0.12
	Phosphorus	0.455-	0.668	1.11-0.78
	Fluoride	3.547	0.016*	3.34-0.53
	Ca/P	1.005	0.361	0.70-0.16
	Surface roughness	5.047	<0.001*	1.82-0.72
Group D	Calcium	1.64	0.162	19.89-4.39
	Phosphorus	2.196	0.079	6.33-0.50
	Fluoride	0.016	0.988	0.53-0.52
	Ca/P	1.034	0.349	0.66-0.28
	Surface roughness	2.724	0.020*	2.10-0.22

*P<0.05-significant. Ca/P: Calcium-phosphorous ratio; CI: Confidence interval

Figure 1: Scanning electron microscope image (at ×2000 magnification) depicting the surface morphology of the demineralized enamel

obliterating the defects, filling up the rods and interrod region, showing an uneven yet more homogeneous surface compared to the control group. The enamel rods and prismatic substance were not discernable, but the areas of calcified deposits comprising of irregularly shaped f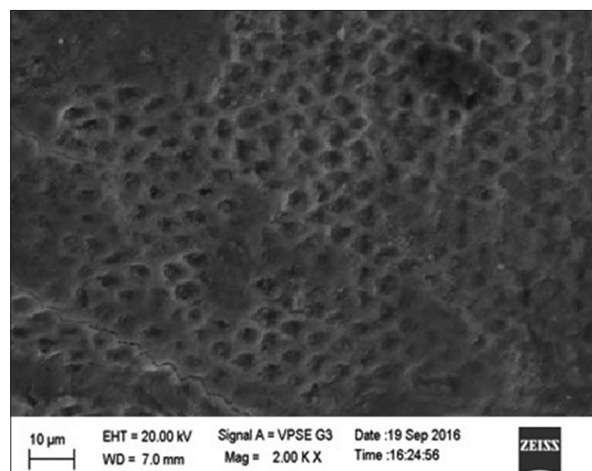luorhydroxyapatite crystals were evident. In all the groups, areas of unfilled defects persisted at 4 weeks remineralization.

Discussion

In this study, a decrease in surface roughness and increase in mineral content were seen in both the fluoride dentifrice groups. The results of the study confirmed the null hypothesis. During remineralization, a superficial layer of fluorapatite is formed. For prolonged anticaries effect, the fluoride needs to be released and deposited slowly and continuously over time, which can occur due to the formation of CaF_2 precipitation on the enamel surface. The calcium fluoride (CaF_2) formed during the conversion of hydroxyapatite to fluorapatite, acts as a reservoir which slowly releases fluoride. This fluoride is available
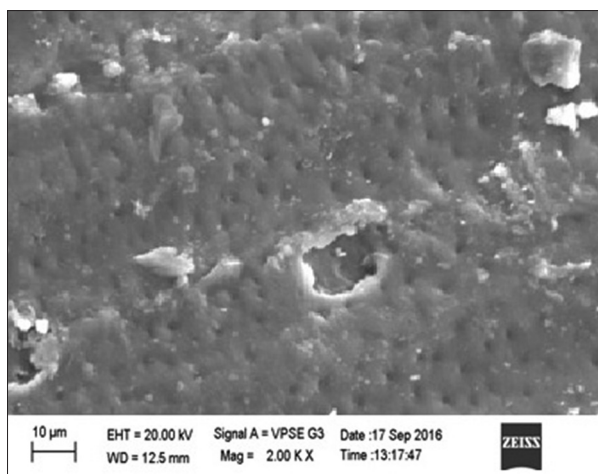
Figure 2: Scanning electron microscope image (at ×2000 magnification) depicting the surface morphology of the enamel of the control group after 4-week remineralization
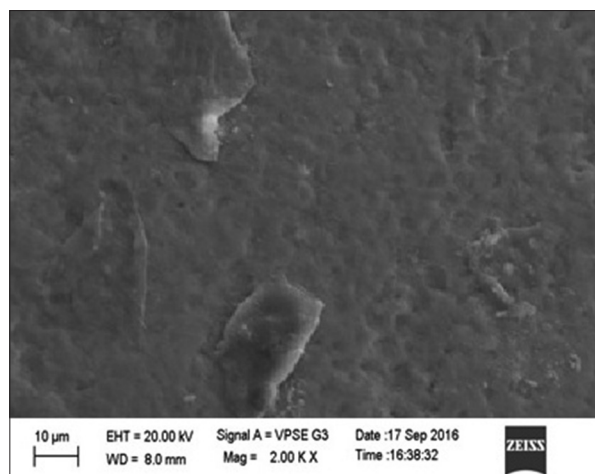

Figure 3: Scanning electron microscope image (at ×2000 magnification) depicting the surface morphology of the enamel of the sodium monofluorophosphate group after 4-week remineralization
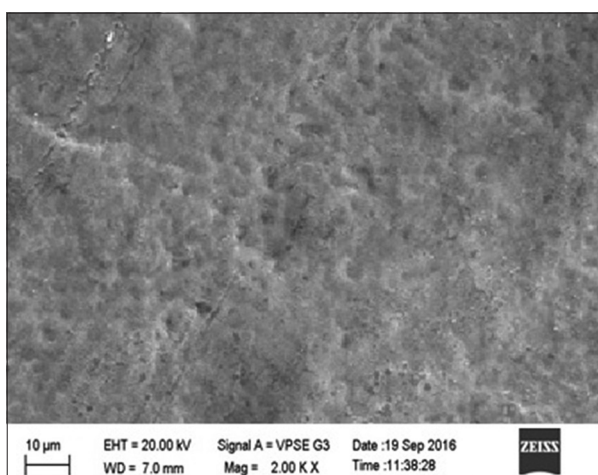

Figure 4: Scanning electron microscope image (at ×2000 magnification) depicting the surface morphology of the enamel of the amine fluoride group after 4-week remineralization
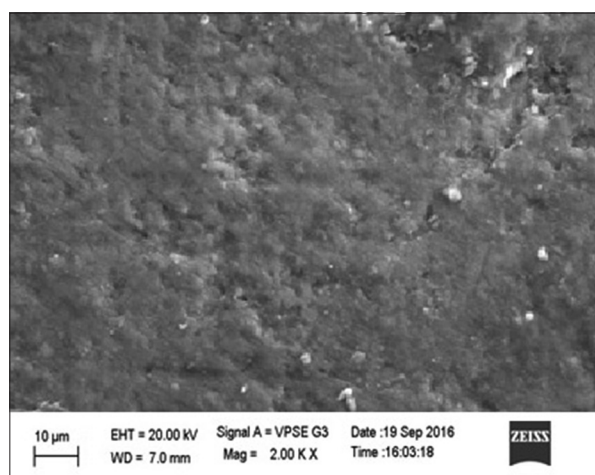

Figure 5: Scanning electron microscope image (at ×2000 magnification) depicting the surface morphology of the enamel of the Anticay® group after 4-week remineralization

for further conversion to fluorapatite, thus enhancing remineralization.[7,20] This explains the significant increase in fluoride content seen in the amine fluoride group after 4 weeks' exposure to the dentifrice. However, it was not significantly different from other dentifrices which was contrary to the results of other studies which claim superior remineralizing effect of amine fluoride-containing dentifrices.[7,8] The difference in results could be due to variation in methodological aspects such as the type of substrate used, characteristics of the artificial enamel lesion formed, and the pH cycling model used.[15] Amine fluoride also has the advantage of antiplaque effect which results in inhibition of bacterial adhesion due to its amine (organic) component. Furthermore, the tensioactive property of the amine component favors the accumulation of fluoride close to the tooth surface providing a sustained fluoride release.[9] The monofluorophosphate in the sodium monofluorophosphate-containing dentifrice directly reacts with hydroxyapatite to form

fluorapatite without the formation of CaF_2.[4] The monofluorophosphate undergoes hydrolysis into phosphate and fluoride ions. The fluoride is incorporated into the apatite crystal to form fluorapatite in an exchange with orthophosphate.[6] The hydrolysis of monofluorophosphate is catalyzed by salivary enzymes which decrease the effectiveness of monofluorophosphate-containing dentifrice in an *in vitro* study design.[4,6] Thus, the lack of superiority of sodium monofluorophosphate dentifrice in remineralization of enamel lesions as per the results of this study should be interpreted with caution.

Ca/P of 1.6 is considered optimal for enamel remineralization. However, in plaque fluid, the Ca/P is approximately 0.3, indicating the presence of an excess of phosphate. Therefore, calcium is considered the major mineral required for remineralization, and an increase in Ca/P is considered favorable for remineralization.[21] In the Anticay® group, the calcium and phosphate ions that are released into

the solution increase the rate of remineralization due to the common ion effect. The sucrose phosphate ions are adsorbed on the enamel surface which helps in decreasing the rate of acid dissolution of hydroxyapatite.[12] However, no significant increase after remineralization could be seen in the Ca/P in the Anticay® group in this study although it showed a maximum increase compared to other groups. There was a decrease in fluoride content after 4 weeks of remineralization. Thus, the soluble calcium and phosphates due to nonavailability of fluoride ions were unable to substantially localize at the tooth surface to produce effective concentration gradients for better remineralization than the other two fluoride-containing dentifrices.[13] Considering that there was no significant difference in the remineralizing effectiveness between the groups, calcium sucrose phosphate with inorganic orthophosphate (Anticay®)-based dentifrice can be an alternative for fluoride dentifrices, especially in children below 6 years of age who are at risk for dental fluorosis due to their tendency to swallow dentifrices.[4,11]

Profilometry is an effective method of measuring the surface roughness of a specimen quantitatively in micrometers. Surface roughness not only affects the esthetic properties of a tooth but also gives an idea about the susceptibility to plaque retention or bacterial adhesion.[22] All the test groups showed a decrease in the surface roughness post-remineralization suggestive of the deposition of minerals into the porous defects[23] as confirmed by SEM images and an increase in the mineral content as seen in the EDAX analysis. A certain amount of remineralization was seen in the control group as there is a tendency for the minerals to diffuse out and supersaturate on the surface during demineralization and also due to immersion of the specimens in the remineralizing solution and artificial saliva.[7]

In this study, sections from the same molar were used in four different groups after random allocation which simulated the split-mouth design, *in vivo*. Such a study design enabled each tooth to serve as its own control and any change observed can be attributed to the dentifrice so that confounding variables such as variations in enamel structure or composition between the teeth of different individuals are eliminated, thus minimizing bias.[24] The pH cycling model used in this study helps to simulate conditions in the oral cavity and mimic the dynamics of the mineral loss and gain during caries process.[4,15] *In vitro* studies have several advantages such as the smaller sample size required, maintenance of high-level scientific control, and lower variability in the samples.[15] However, the study has its limitations due to the design. The surface aprismatic layer was removed with fine-grit polishing to create a more uniform surface, required for the assessment of surface roughness and mineral content *in vitro*, which may not mimic the clinical situation.[25] Furthermore, *in vitro* studies cannot totally replicate the normal oral environment as the enzyme systems required for the hydrolysis of sodium monofluorophosphate are not available; the antiplaque effect of amines does not play a role in *in vitro* remineralization; and the variation in the quantity and quality of saliva in the oral cavity at different points of time is not simulated.[15] Hence, results of this study should be further confirmed through *in vivo* studies.

Conclusions

Within the limitations of the study, it can be concluded that sodium monofluorophosphate, amine fluoride, and calcium sucrose phosphate with inorganic orthophosphate (Anticay®)-based dentifrices are equivocal in their effectiveness of remineralizing early enamel lesions.

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Conflicts of interest

There are no conflicts of interest.

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