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ORIGINAL ARTICLE

Comparison of remineralization by fluoride varnishes with and without casein phosphopeptide amorphous calcium phosphate in primary teeth

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ABSTRACT

Objective: To compare MI (5% NaF with 2% CPP-ACP) and Prevident (5% NaF) varnishes in remineralizing caries-like lesions in primary teeth regarding calcium and phosphate enamel content and lesion depth.

Material and methods: Caries-like lesions were created in 48 primary teeth which were divided into 2 halves; one left untreated (control) and the other half treated with MI or Prevident varnishes. Calcium and phosphate content was assessed using energy dispersive X-ray spectrometer and reduction in lesion depth was assessed using polarized light microscopy. Demineralization and remineralization values in each group were compared using paired *t* test and percentage change between groups was compared using *t* test and Mann Whitney *U* test.

Results: A greater percentage increase of calcium was observed in MI than Prevident specimens (median = 8.97 and 2.67, $p < .0001$), with greater calcium phosphate ratio percentage increase (median = 28.96 and 7.40) and phosphate percentage reduction (median = 15.5 and 4.51). The mean (SD) percentages reduction in lesion depth in the MI varnish was significantly greater than in Prevident varnish (44.41 (7.12) and 22.73 (9.35), $p < .0001$).

Conclusions: MI varnish had better remineralization effect in primary teeth than Prevident varnish in terms of higher mineral content and shallower lesion depth.

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Introduction

Caries progression depends on a dynamic process characterized by alternating periods of demineralization and remineralization. Demineralization can be reversed if the pH is neutralized and there were sufficient calcium and phosphate ions in the presence of fluoride in the immediate environment. This enables the rebuilding of partly dissolved apatite crystals through remineralization [1] with the remineralized crystals being less acid soluble than the original mineral [2].

Fluoride varnish contains concentrated fluoride (5% NaF, 22.600 ppm F). It adheres to tooth surface in the presence of saliva, increasing the time fluoride is in contact with the tooth [3]. Varnishes are relatively safer [4], more acceptable and easier to apply than other topical fluorides [5]. Fluoride varnish was reported to be effective in reducing caries lesion depth in primary teeth [6].

Dairy products were found to have cariostatic effect due to the chemical effect of casein phosphopeptides (CPP) complexed with calcium. CPP has a remarkable ability to stabilize calcium and phosphate as nanoclusters of ions in solution forming casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) [7] that diffuses into enamel to repair demineralized areas with proven effect on increasing the percentage of calcium in enamel after its application [8]. It had higher remineralization potential than Durashield (500 ppm sodium fluoride) in primary teeth when used for 30 days in an *in vitro* model [9].

Casein phosphopeptides bind fluoride as well as calcium and phosphate, and thus can stabilize calcium fluoride phosphate as soluble complexes [10]. Compared to CPP-ACP, fluoride containing CPP-ACP (CPP-ACPF) resulted in better enamel remineralization in permanent teeth in a lab study [11] and *in vivo* [12]. An *in vitro* study also showed a higher remineralization potential of CPP-ACPF compared to CPP-ACP in primary teeth [13].

A better remineralizing potential of CPP-ACPF was also reported compared to sodium fluoride alone in bovine enamel [14] and permanent enamel [15]. Another study comparing CPP-ACPF (containing 0.9 mg/gm fluoride) versus 0.05% sodium fluoride mouth rinse in permanent teeth showed no difference in calcium/phosphorus ratio (Ca/P ratio) with shallower enamel lesions in CPP-ACPF compared to sodium fluoride rinse [16]. Fluoride varnishes containing CPP-ACP are now available as MI varnish (5% NaF and 2% CPP-ACP) (GC Company, Instructions for use of MI varnish, 2017). MI varnish was reported to be more effective than 5%

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sodium fluoride varnish in permanent teeth regarding reducing caries lesion depth around orthodontic brackets [17] and releasing higher levels of calcium, phosphate and fluoride ions [18].

Compared to the studies conducted on the remineralization potential of MI varnish versus sodium fluoride varnishes in permanent teeth, fewer studies investigated this potential in primary teeth. Such a potential may provide an additional tool for controlling and preventing early childhood caries in younger children. The hypothesis of the study was that a fluoride varnish containing CPP-ACP (MI varnish) would be better than a varnish containing only fluoride (Prevident varnish) in remineralizing primary teeth. The aim of this study was to compare the effectiveness of MI varnish and Prevident varnish in reducing the depth of caries-like lesions in primary teeth through minerals deposition.

Materials and methods

An experimental *in vitro* study was conducted in the labs of the Department of Oral Biology, Faculty of Dentistry and Department of Geology, Faculty of Science and the clinic of the Department of Pediatric Dentistry and Dental Public Health, Faculty of Dentistry, Alexandria University. Approval to conduct the study was obtained from the Research Ethics Committee of the Faculty. The study has been conducted in accordance with the Declaration of Helsinki.

Sample size was estimated based on assuming alpha error = 5% and beta error = 20%. We used the estimates of lesion depth reduction of F only varnish (mean (SD) = 26.8 (12.6)) and F/CPP-ACP combination (mean (SD) = 35.6 (14.9)) compared to negative control (mean (SD) = 51.2 (24.4)) from Pulido et al. [19] and calculated mean percent reduction after fluoride only varnish = 47.7% and after MI varnish = 30.5% with pooled SD = 14. The calculated sample size was 11, increased to 12 per group to allow for processing errors.

We used 48 maxillary extracted/exfoliated anterior primary teeth collected from the outpatient clinics of the Faculty of Dentistry, Alexandria University. Teeth were examined using a magnifying lens to ensure that they were free from caries, cracks or developmental defects. Teeth were cleaned with a brush and fluoride-free pumice and stored in deionized water at room temperature. They were dried and 4 × 4 mm windows were created to expose enamel areas after covering the remaining surface with sticky tapes. Teeth were immersed in demineralizing solution (2.2 mM calcium chloride, 2.2 mM potassium dihydrogen phosphate, 0.05 M acetic acid and 1 M potassium hydroxide to adjust the pH to 4.4.) [20] for 4 days to create subsurface enamel caries-like lesions [21] followed by rinsing and storage in 100% humidity. Each tooth was sectioned longitudinally in a labiolingual direction through the center of the window into two halves. One half was treated with the remineralizing agent and the other half was left untreated and served as control. Each half was considered a specimen.

The 48 teeth (96 specimens) were randomly distributed into two groups; one where the test half was treated with MI varnish; 5% NaF varnish containing 2% Recaldent™ (CPP-ACP) (GC America Inc., IL) and another group were the test half was treated with Prevident varnish; 5% NaF varnish (Oral Pharmaceuticals Subsidiary of Colgate-Palmolive Company, NY). A thin, uniform coating of the test varnish was applied and left in contact with the tooth surface for 4 h.

The varnishes were removed from test specimens using a cotton swab soaked with acetone without damaging the enamel surface [22]. The specimens were rinsed and stored in artificial saliva (500 ml distilled water, 20 g xylitol, 1.2 g potassium chloride, 0.843 g sodium chloride, 0.051 g magnesium chloride, 10.00 g sodium carboxy methyl cellulose, 20 ml stock solution of 1% tricalcium phosphate and 0.05 M sodium hydroxide added to adjust the pH to 7.0) [23] for 24 h. All specimens were exposed for pH cycling model over a period of 10 days to cycles of demineralization and remineralization [20,22] including 6 h of demineralization (pH = 4·4) followed by 18 h of remineralization (pH = 7·0) using a remineralizing solution (1.5 mM calcium chloride, 0.9 mM sodium dihydrogen phosphate and 0.15 M potassium chloride used to adjust the pH to 7.0) [20] then incubated at 37 °C.

Twenty-four specimens and their corresponding controls were evaluated using polarized light microscope (Olympus America Inc, U.S.A) while the remaining 24 specimens with their corresponding controls were analyzed using Energy dispersive X-ray spectrometer (EDX) (Jeol JSM-5300, U.S.A).

Using polarized light microscopy [24], longitudinal ground enamel sections of 15 μm thickness were examined to measure lesion depth. Polarized light microscope can show different birefringence. The minerals in enamel refract polarized light in rays with 2 different amplitudes and refractive indices related to two planes of transmission (birefringence). The positive birefringence refers to the ray with the smaller amplitude (the slower ray) and the negative birefringence refers to the ray with the bigger amplitude (the faster ray). Respectively, these can be seen as dark, brown color and bluish green color [24]. Normal enamel was identified through its prismless surface layer, a dark continuous ribbon over the surface with striae of Retzius and alternative Hunter-Shreger bands (Figure 1). Demineralized enamel

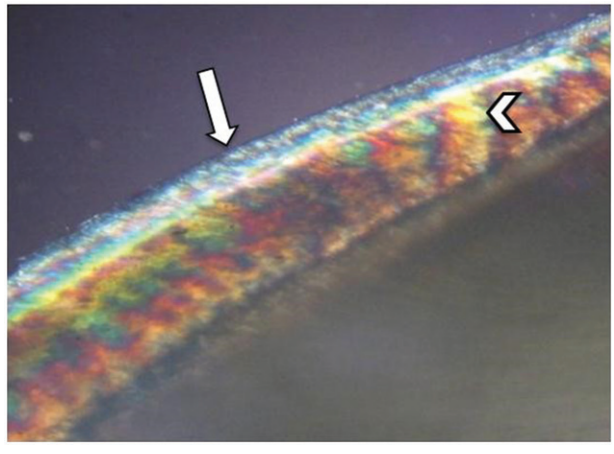

Figure 1. Polarized light photomicrograph of longitudinal ground normal enamel section with prismless enamel layer (arrow) and Hunter-Shreger bands (arrow head), magnification ×40.

showed different zones (deep to superficial); a translucent zone, a dark zone, body of lesion and surface zone with positive birefringence (dark color) and loss of striae of Retzius and Hunter-Shreger bands (Figure 2). Remineralized enamel showed negative birefringence (greenish blue color) in MI varnish group (Figure 3) and in Prevident varnish group (Figure 4). Photomicrographs were obtained using a digital camera to measure lesion depth through an eyepiece-graduated lens micrometer with magnification = ×40. The mean depth in micrometers (μm) was the average of three lines: one on each side of the lesion and a third one in the center [25]. Examiner consistency was checked by repeated measurement of 10 specimens and comparing the duplicates using paired *t* test where no significant difference was found between these values.

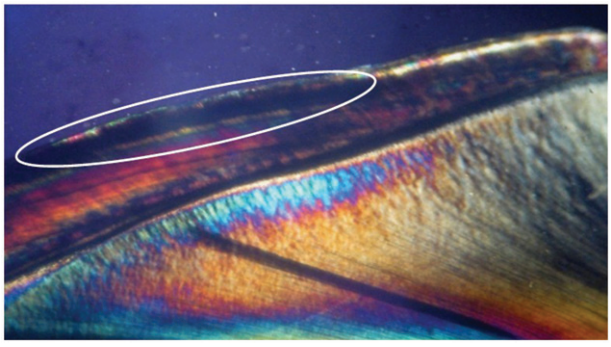

Figure 2. Polarized light photomicrograph of longitudinal ground section of demineralized enamel showing dark demineralized band of enamel, magnification ×40.

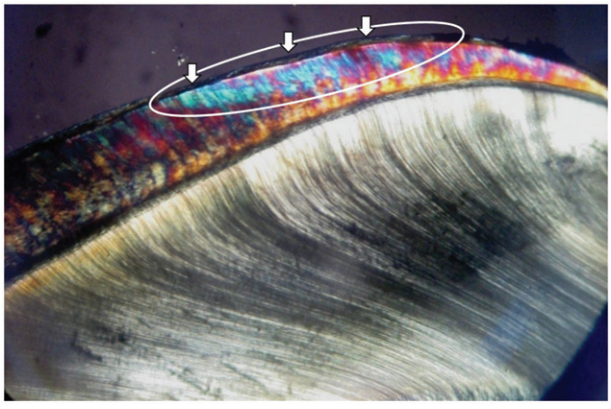

Figure 3. Polarized light photomicrograph of a longitudinal ground section treated with MI varnish showing well-defined negative birefringent surface zone and circumscribed remineralization of the body of the lesion, magnification ×40.

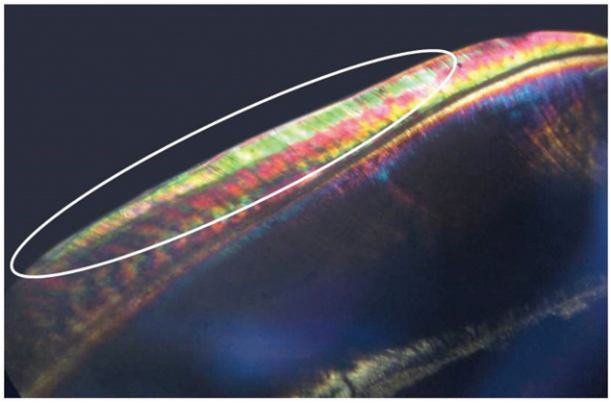

Figure 4. Polarized light photomicrograph of a longitudinal ground enamel section treated with Prevident varnish showing homogenous remineralization of the caries like lesion, magnification ×40.

EDX examination conducts quantitative micro analysis of the composition of the specimens by measuring the characteristic re-emission from each constituent by using the scanning electron microscope [26]. The specimens were dehydrated and mounted on copper stubs and exposed to EDX. Distribution of calcium (Ca) and phosphorus (P) in atomic % was determined by peaks on the resulting software graph with corresponding readings (Figure 5). Ca/P ratios were calculated.

Statistical analysis

Two outcome variables were studied; lesion depth and content of calcium and phosphate in addition to Ca/P ratio. In each group, the values for these variables at demineralization and remineralization were compared using paired *t* test. Percent change of the outcome variables in the test half relative to the control half was calculated as follows: [(value in test half − value in control half)/value in control half] × 100. Normality was checked using Kolmogorov Smirnov test. Mean percentage reduction in lesion depth was compared between the two groups using *t* test whereas median percent change of Ca, P, Ca/P ratio was compared using Mann Whitney *U* test. Significance was set at the 5% level. SPSS version 20 was used for statistical analysis.

Results

Table 1 shows that the mean (SD) lesion depth in the MI varnish group was reduced from 252.78 μm (10.86) at demineralization to 140.28 μm (16.60) after remineralization, percentage reduction = 44.41%. In the Prevident varnish group, the depth was reduced from 242.36 μm (15.27) to 188.19 μm (30.46), percentage reduction = 22.73%. The reduction in lesion depth in each group was statistically significant ($p < .0001$) with a statistically significant difference between the two groups ($p < .0001$).

Table 2 shows that calcium and Ca/P ratios significantly increased from demineralization to remineralization, whereas there was a significant reduction in phosphate in the two groups ($p < .0001$). The percentage increase in calcium was significantly higher in MI varnish than in Prevident (mean- = 8.97 and 2.67, $p < .0001$). The percentage decrease in phosphate was significantly higher in MI varnish than in Prevident (mean = 15.5 and 4.51, $p < .0001$). The percentage increase in Ca/P ratio was significantly higher in MI varnish than in Prevident (mean = 28.96 and 7.40, $p < .0001$).

Discussion

The hypothesis of the present study was supported by the results. MI varnish was more effective in increasing calcium

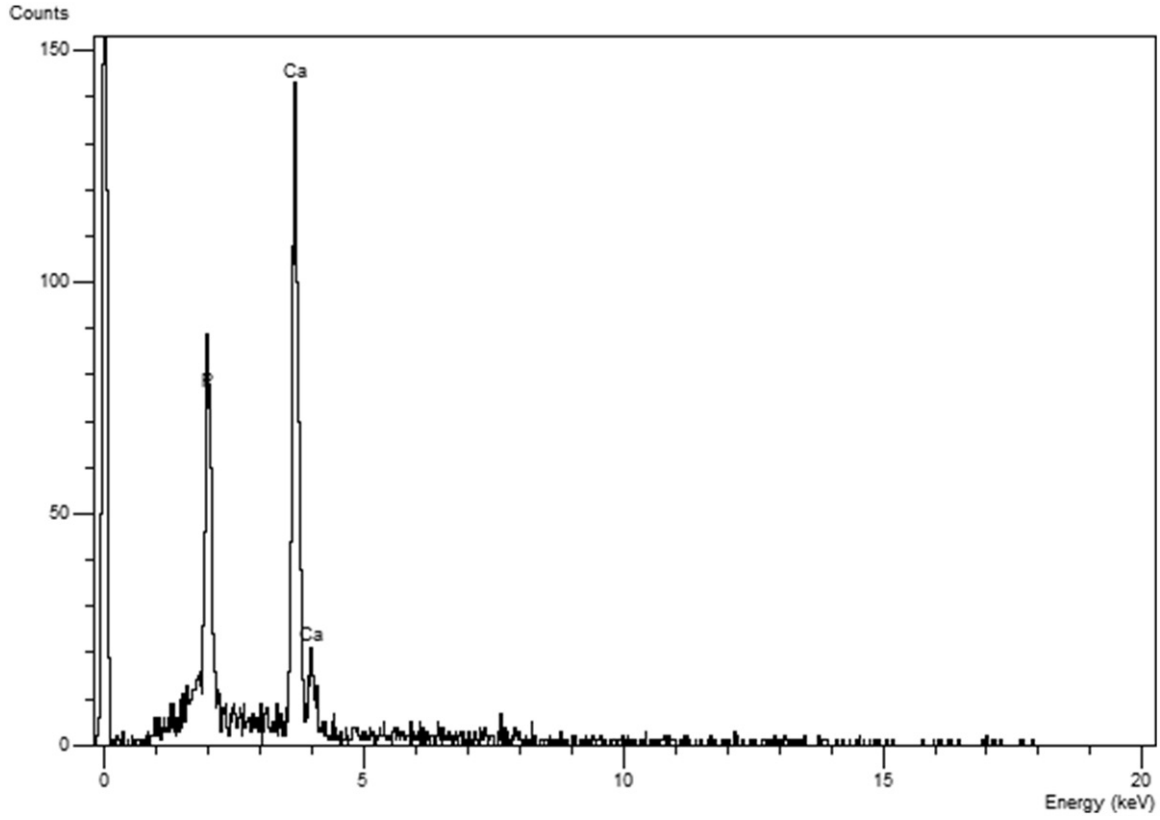

Figure 5. Analysis of Ca and P using EDX.

Table 1. Lesion depth at demineralization, after remineralization and percentage reduction in MI and Prevident varnishes.

	Lesion depth (μm) Mean (SD)	
	MI varnish	Prevident varnish
Demineralized	252.78 (10.86)	242.36 (15.27)
Remineralized	140.28 (16.60)	188.19 (30.46)
p Value of paired *t* test (demineralization–remineralization	<.0001*	<.0001*
% reduction	44.41 (7.12)	22.73 (9.35)
p Value of independent samples *t* test (between the two groups)	<.0001*	

*Statistically significant at $p \leq .05$.

and Ca/P ratio and inducing greater reduction in lesion depth after remineralization compared to Prevident varnish. Our study also showed that increasing calcium and Ca/P ratio with concomitant reduction in phosphate was associated with lesion depth reduction and this is why MI varnish had greater effect than Prevident varnish. Our findings have implication for the arrest of incipient caries lesions in primary teeth. The present study filled a gap in knowledge by adding to the limited evidence on the difference in action of these two varnishes in primary teeth by comparing their effect on mineral content and lesion depth.

We used MI varnish which contains a high fluoride concentration. This evaluates the potential for maximum protection against caries based on the concept of dose–response effect of fluoride on the demineralization–remineralization process [19].

Our results showed that in both groups, the varnish-treated test halves had shallower lesions than the untreated control halves, with significantly greater reduction caused by the MI varnish than Prevident varnish. Our results agree with previous studies showing that MI varnish resulted in significantly shallower lesions than the 5% NaF fluoride varnish whether in primary teeth [27], bovine enamel around orthodontic bands [17] or enamel slabs [18]. The greater effect can be explained by the synergistic effect of fluoride, calcium and phosphate in MI varnish offering greater remineralization capacity than the fluoride varnishes only.

It should be noted that in these previous studies, MI varnish was left on enamel for longer periods ranging from overnight [18], 24 h [28] to 28 days [17] which might have released more minerals from MI varnish into enamel and produced more remineralization effect. By contrast, the control of early childhood caries was the rationale behind our study. For that purpose, the varnish would be applied on primary teeth of young children and shorter duration would therefore be more realistic. We left both varnishes in contact with enamel for 4 h following the manufacturers' instructions. The ability of MI varnish to produce shallower lesions in spite of this maybe due to this 4 h duration representing adequate duration beyond which no significant increase in minerals deposition would occur.

Some previous studies – in contrast to ours – reported lower effect on caries lesion depth of MI varnish compared to varnishes containing only fluoride [27,29,30]. A study reported that MI varnish had three times as much fluoride release as 5% NaF Prevident varnish but about one third the effect on uptake leading to equal effects of the two varnishes on surface hardness [27]. In that study, however, a different methodology used surface hardness as a proxy for

Table 2. Calcium and phosphate enamel content and Ca/P ratio at demineralization, after remineralization and percentage change in MI and Prevident varnishes.

	Calcium		Phosphate		Ca/P ratio	
	MI varnish	Prevident varnish	MI varnish	Prevident varnish	MI varnish	Prevident varnish
Demineralized: mean (SD)	64.68 (1.86)	60.59 (2.38)	35.28 (1.85)	39.42 (2.40)	1.84 (0.15)	1.55 (0.16)
Remineralized: mean (SD)	70.68 (3.40)	62.50 (2.51)	29.3 (3.39)	37.52 (2.49)	2.45 (0.41)	1.68 (0.18)
p of paired t test	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*
% Change: median (min/max)	8.97 (3.74/17.98)	2.67 (0/5.86)	−15.50 (−31.40/−6.70)	−4.51 (−8.44/0)	28.96 (11.19/70.24)	7.40 (0/15.49)
p of MWU test	<.0001*		<.0001*		<.0001*	

MWU test: Mann–Whitney U test.
*Statistically significant at $p \leq .05$.

remineralization and this may explain the difference between their results ad ours. Another study reported less effect of MI varnish on lesion depth and mineral loss in permanent teeth than 5% NaF Duraphat varnish [29]. The differences between these studies and ours might be attributed to mainly different methodologies and to a lesser extent to the greater release of inorganic phosphate by MI varnish that may reduce calcium fluoride formation and decrease the bioavailability of fluoride needed for remineralization [30].

The present study showed a reduction in phosphate content in enamel after remineralization in both groups. This disagrees with a study that reported an increase in phosphate content after remineralization of bovine enamel specimens [31]. This difference may be attributed to the chemical structure of primary enamel which differs from permanent/other types of enamel [32].

Our study showed that within the time that varnishes are expected to be in contact with enamel in clinical settings, MI varnish had greater effect on reducing caries-like lesion depth than Prevident varnish with higher minerals deposition. One of the limitations of the present study was the absence of the natural environment of the oral cavity. The pH cycling model provided a controlled environment that helped in focused assessment of related variables independently from each other- which is difficult to achieve in the oral cavity. However, it did not entirely simulate the complex oral conditions where pH fluctuates frequently and the rate of salivary flow may affect the clearance of varnishes or some of their constituents. We did not measure the concentration of fluoride in enamel similar to calcium and phosphate and this needs to be addressed in future studies so that it becomes possible to have a more comprehensive understanding of the interplay between the three minerals affecting caries progression.

Disclosure statement

No potential conflict of interest was reported by the authors.

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