

EFFECT OF FLUORIDE VARNISH CONTAINING XYLITOL-COATED CALCIUM AND PHOSPHATE ON THE REMINERALIZATION OF CARIES LIKE LESIONS IN PRIMARY TEETH

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ABSTRACT

INTRODUCTION: Embrace™ Varnish is a new fluoride varnish which contains xylitol-coated calcium and phosphate (XCP). This combination leads to increase the release time of fluoride in this varnish.

OBJECTIVES: To compare the remineralizing effect of Embrace™ varnish with Profluoride® varnish on caries-like lesions in primary teeth regarding lesion depth and calcium and phosphate enamel content.

MATERIALS AND METHODS: Forty eight anterior primary teeth were coated with nail varnish, leaving squares of 4x4 mm then sectioned longitudinally in a labiolingual direction into two equal halves. Each half was considered as a specimen (96 specimens). Caries-like lesions were created in all specimens. Specimens were divided into two groups. group I (n=48) was subdivided into subgroup IA (n=24) treated with Embrace™ varnish and subgroup IB (n=24) served as control, while group II (n=48) was subdivided into subgroup IIA (n=24) treated with Profluoride® varnish and subgroup IIB (n=24) served as control. Specimens were subjected to pH cycling then they were examined with polarized light microscopy and energy dispersive X-ray spectrometer.

RESULTS: No significant difference in mean percent reduction in lesion depth following treatment with Embrace™ varnish and Profluoride® varnish (49.51 and 45.11, respectively) at $P= 0.58$. Greater significant of calcium phosphate ratio was found for Embrace™ varnish (2.25 ± 0.43) than Profluoride® varnish (1.65 ± 0.05) at $P< 0.0001$. Significance was set at the 5% level.

CONCLUSIONS: Embrace™ varnish had no significant difference in mean percent reduction in lesion depth than Profluoride® varnish, while it had a better remineralization effect than Profluoride® varnish in terms of higher mineral content.

KEYWORDS: Embrace™ varnish, Xylitol coated Calcium and Phosphate (XCP), fluoride varnish, polarized light microscopy, energy dispersive x-ray spectrometer.

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INTRODUCTION

Dental caries prevalence has decreased in many developed countries. However, it is still a considerable public health problem among children (1). Caries process begins with enamel dissolution when pH drops below 5.5 due to exceeding of demineralization rate than remineralization one (2). Incipient carious lesion (ICL) is the earlier stage of enamel caries, with mineral loss from subsurface enamel, while surface enamel remains intact (3). Application of fluoride varnish to white spot lesions has been reported to be effective in reversing these lesions (4).

Fluoride ions enhance the remineralization of incipient carious lesions (5). Fluoride varnish is the most acceptable fluoride delivery vehicle due to the extended time of contact between fluoride and tooth surfaces. So, this avoids the direct loss of fluoride after application (6). An acceptable amount of calcium and phosphate ions which is supplied into the topical fluoride therapy has an essential role in caries prevention. So, introduction of

high concentration of calcium phosphate remineralizing systems were mandatory (7).

Embrace™ Varnish is a new generation of fluoride varnish which has been introduced through combination of Xylitol- coated Calcium and Phosphate (XCP) in a resin matrix. This combination leads to increase fluoride release time of this varnish. The xylitol is dissolved by the saliva leading to the release of calcium and phosphate ions to react with the fluoride ions and form fluoroapatite on the enamel surface (8).

According to Rao A, Malhotra N, 2011 (9), combination of xylitol with sodium fluoride can enhance remineralization of the demineralized subsurface enamel by assisting calcium ions penetration and deposition.

Milburn et al, 2015(8), compared Embrace™ varnish with the currently marketed topical fluoride varnishes regarding the quantity and rate of fluoride release from the enamel of human molars. Embrace™ varnish showed the greatest initial fluoride release.

Mohd Said et al, 2017(7), compared the remineralization effect of ACP, CPP-ACP, fTCP with Embrace™ varnish on artificial enamel caries of human molars using Knoop surface microhardness (KHN) and Transverse Microradiography (TMR). It was concluded that the remineralization effect of Embrace™ varnish was significantly lower than the sodium fluoride varnish.

Jain et al, 2019 (10), assessed the remineralization potential of Fluoritop-SR (5% NaF), Clinpro XT (resin modified glass ionomer-based calcium phosphate) and Embrace™ fluoride varnishes using scanning electron microscopy and energy dispersive X-ray. It was demonstrated that the use of Embrace™ varnish may result in better remineralization of early enamel lesions when compared to 5% sodium fluoride varnish.

These conflicting results suggest the need for further studies to clarify the role of Embrace™ varnish in remineralization of artificial enamel caries of primary teeth. So, the purpose of this in vitro study was to evaluate its remineralization effect and compare it with sodium fluoride varnish on a caries like enamel lesions in primary teeth. The null hypothesis was that there would be no difference in the remineralization potential among topical fluoride varnishes that were used on the primary teeth.

MATERIALS AND METHODS

Study design

The study was an experimental in vitro comparative study with two parallel arms; test and control.

Sample size estimation

Ethics committee of Faculty of Dentistry Alexandria University has approved this in vitro experimental study (IORG 0008839). The minimal sample size was calculated based on a previous study aimed to evaluate the effect of fluoride varnishes on remineralization of primary enamel lesions (11). Calculation resulted in a standardized effect size (δ) of 1.7828 of percent reduction of carious lesion after remineralization (primary outcome) that resulted in a minimum required sample size of 40 specimens per group (number of groups = 2). Total sample size needed = 80 specimens (12). As statistically significant with 80% power and at a significance level of 95% (alpha error accepted = 0.05). Sample size was increased to 48 specimens per group (total sample size= 96 specimens) (20% increase) to control for withdrawal (attrition) bias (13). The sample size was calculated using IBM SPSS Sample Power 3.0.1. (2010)(14).

Tooth inclusion and exclusion criteria

Forty eight extracted/exfoliated anterior primary teeth were collected from the outpatient clinics of the Faculty of Dentistry, Alexandria University. After thoroughly cleaning the teeth, they were examined by a magnification lens to ensure that these teeth met the inclusion criteria of having no caries, cracks or developmental defects.

Specimens preparation

The teeth were cleaned with fluoride free pumice then washed with distilled water and air-dried. A 4×4 mm square of self-adhesive tapes were stuck at the center of the middle third of the labial surface of each tooth. Acid-

resistant nail varnish was used to coat each tooth surface. The adhesive labels were then removed to expose only a small window of enamel (15).

Sectioning of the teeth

Each tooth was sectioned longitudinally in a labiolingual direction through the center of the window with a diamond disc (911pf-220-0.25 by Diatech Swiss Dental Instruments) mounted on a straight hand piece to obtain two equal halves. Each half was considered as a specimen. One half was treated with the remineralizing agent while the other half was left untreated to serve as a control (15).

Caries-like lesion formation

Subsurface enamel lesions were produced by immersing all teeth in demineralizing solution for 4 days. The demineralizing solution (solutions were prepared in the Labs of Faculty of Pharmacy, Alexandria University) was prepared by adding 2.2 mM Calcium chloride (Ca Cl₂), 2.2 mM Potassium dihydrogen phosphate (NH₂PO₄), 0.05M Acetic acid (CH₃ COOH), 1 M Potassium hydroxide KOH was used to adjust the pH to 4.4 (16).

Grouping

Forty eight teeth (96 specimens) were randomly divided into two main groups (I and II) by using computer generated list of random numbers. Group I consisted of 48 specimens which were subdivided into two subgroups: subgroup IA (24 specimens) which served as a test and was treated with Embrace™ Varnish and subgroup IB (24 specimens) which served as a control and was left untreated. Group II consisted of 48 specimens which were similarly subdivided into two subgroups: subgroup IIA (24 specimens) which served as a test and was treated with Profluoride® Varnish, while subgroup IIB (24 specimens) which served as a control and was left untreated.

Fluoride varnish treatment

As a manufacture instruction, a thin, uniform coating of the test varnishes were applied. Embrace™ varnish (Pulpdent® Corporation, Watertown, USA.) was applied for specimens of subgroup IA, while Profluoride® varnish (VOCO GmbH, Cuxhaven, Germany) was applied for specimens of subgroup IIA. Subgroups IB and IIB which served as control were left untreated. All specimens were then immersed for 6 h in artificial saliva which was prepared by adding distilled water (500 ml), Potassium chloride (1.2 g), Sodium chloride (0.843 g), Magnesium chloride (0.051 g), Sodium hydroxide (0.05 M) added to the mixture to have a pH= 6.8, Stock solution of tricalcium phosphate (TCP) 1% (20 ml) (16). Finally, the varnishes were removed from test specimens by cotton swab soaked with acetone to prevent damaging of the enamel surface (17). All specimens were rinsed with deionized water then pH cycles were started.

pH-cycling model

Seven days of pH cycling model was used for all the specimens. Cycles of demineralization and remineralization including 6 h of demineralization (pH=4.4) followed by 18 h of remineralization (pH=7.0). The remineralizing solution was prepared by mixing 1.5 mM CaCl₂, 0.9 mM NaH₂PO₄, 0.15 M KCL used to adjust the pH to 7.0 then incubated (ZRD A7080 Human-lap korea) at 37 °C (18).

Polarized light microscope

The specimens were evaluated by the polarized light microscope (Olympus America Inc, USA) both qualitatively (histologically) and quantitatively (mean lesion depth). Each specimen was manually ground on a wet glass plate with aluminum oxide (Al_2O_3) powder with different granulations to produce the thin longitudinal ground sections of about 100 μm thickness. An electronic digital caliper (Starrett 727; Brazil) was used to measure the thickness of the ground sections (19). Each ground section was washed under running water then passed in ascending grades of alcohol (50, 70, 90 and 100%). Xylol was used for clearance. Canada balsam was used to hold the specimen in place between the slip cover and the glass slide (20). Depth measurements were done using (image J.46) software. Photomicrographs were obtained using a digital camera to measure lesion depth with magnification= $\times 40$. The mean depth of the enamel lesion of each specimen was measured in micrometers (μm) by averaging of three lines: one at each side and one at the center of the lesion within the subsurface of the lesion body, perpendicular to the outer layer of the enamel surface and extending to the translucent band (Figure 1) (15, 21). Additional ground section was prepared from sound primary enamel which acts as a reference section for comparison and was not included in the study. In the current study, histological evaluation involved describing the interpretation of enamel birefringence. Demineralized enamel showed positive birefringence (dark color) and loss of striae of Retzius and Hunter-Shreger bands, while remineralized enamel showed negative birefringence (greenish blue color) (22).

Intra-examiner reliability

Intra-examiner reliability was assessed by measuring the lesion depth for 10 specimens twice by the same examiner with three days period apart and the agreement of Intra-class correlation coefficient (ICC) was 0.78 (23).

Energy Dispersive X-ray Spectrometer (EDX)

EDX examination used the scanning electron microscope (Jeol JSM-5300, U.S.A) (Figure 2) for analyzing the mineral component of the specimens quantitatively by evaluating the characteristic re-emission from each component. Dehydration of the specimens were performed by immersion them in ascending grades of ethyl alcohol freshly prepared by diluting absolute alcohol with distilled water as follows (15,24):

30 % for 7.5 minutes, then in a fresh solution of the same concentration for another 7.5 minutes.

50% for 7.5 minutes, then in a fresh solution of the same concentration for another 7.5 minutes.

70% for 7.5 minutes, then in a fresh solution of the same concentration for another 7.5 minutes.

After the completion of dryness of the specimens in the fresh air, they were coated with a conductive carbon layer using a JEOL JEE-4X Vacuum Evaporator then mounted on a copper stub and exposed to the EDX (25). The distribution of calcium (Ca) and phosphorus (P) in atomic % was determined by peaks on the resulting software graph with equivalent readings (Figure 3). 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. Normality was checked using descriptive statistics, plots (histogram and box plot) and Shapiro Wilk test. Lesion depth and mineral contents were presented using mean and standard deviation. Since all data was normally distributed, therefore, comparisons between Embrace™ varnish and Profluoride® varnish regarding the lesion depth, elemental contents and percent change were done using independent t test. Whereas, differences between each varnish and its control group regarding the same variables were tested by paired t test. Percent reduction in lesion depth in each test group relative to its control group was calculated according to the following formula: $[(\text{values in test group} - \text{values in control group}) / \text{values in control group}] \times 100$. Significance level was set at $P < 0.05$. Data was analyzed using Statistical Package for the Social Sciences (IBM SPSS) statistical software (version 25).

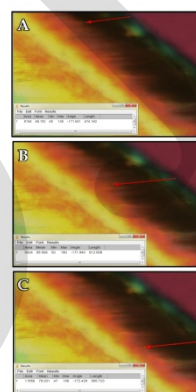


Figure (1): Polarized light photomicrographs showing the use of the ruler to measure the lesion depth, magnification X40. **A&C:** Sides of the lesion. **B:** Center of the lesion.



Figure (2): Energy dispersive X-ray spectrometer

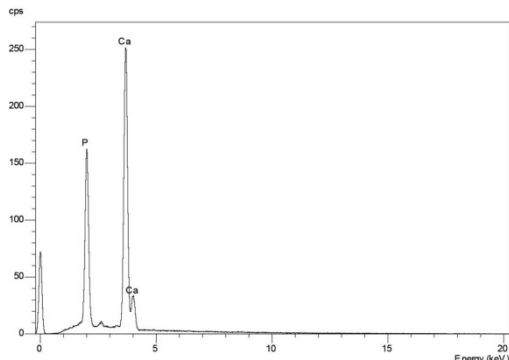


Figure 3: Elemental analysis of % weight of Ca and P contents.

RESULTS

Histological (Qualitative) observation using Polarized light microscope

Normal Enamel

Prismless surface layer of sound primary enamel was identified through a continuous uniform sheet extending all over the enamel surface with alternating light and dark bands of Hunter-Schreger which denote the normal course of enamel rods (Figure 4).

De/Remineralized enamel

Demineralized enamel showed dark bands or laminations within the body of the lesion. Also, it showed a relatively high degree of positive birefringence with loss of the typical striae of Retzius and Hunter-Schreger bands within the body of the lesion (Figure 5: A&C). Remineralized enamel showed negative birefringence in Embrace™ varnish group (Figure 5, B) and in Profluoride® varnish group (Figure 5, D).

Lesion depth (Quantitative) evaluation

The mean lesion depth values were recorded at demineralization and after remineralization. Changes in the lesion depth values are shown in Table 1. There was a significant difference between Embrace™ varnish group (subgroup IA) and its control (subgroup IB) in the reduction of lesion depth ($P < 0.0001$). Also, the mean (SD) lesion depth of Profluoride® varnish (subgroup IIA) shows significant difference with its control (subgroup IIB) ($P < 0.0001$). The mean (SD) lesion depth in the Embrace™ varnish group was reduced from $644.67 \pm 188.93 \mu\text{m}$ at demineralization to $310.75 \pm 111.71 \mu\text{m}$ after remineralization, percentage reduction $49.51 \pm 17.81\%$. In the Profluoride® varnish group, the lesion depth was reduced from $558.58 \pm 188.37 \mu\text{m}$ to $318.17 \pm 176.49 \mu\text{m}$, percentage reduction $45.11 \pm 20.53\%$. There was no significant difference between the two groups in the reduction of lesion depth ($P = 0.58$).

Energy Dispersive X-ray Spectrometer Results

The calcium and Ca/P ratios were recorded for both groups at demineralization and remineralization stages. Changes in the values of Ca, P and Ca/P ratios are shown in Table 2. There was a higher significance in the mean Ca content and mean Ca/P ratio between both tested varnishes (Embrace™ and Profluoride varnishes) with their control subgroups (subgroup IB & subgroup IIB) at ($P < 0.0001$). While There was a significant lower in the

mean P content between both tested varnishes (Embrace™ and Profluoride® varnishes) with their control subgroups (subgroup IB & subgroup IIB) at ($P < 0.0001$). The increase in calcium was significantly higher in Embrace varnish than in Profluoride® (mean \pm SD = 64.80 ± 3.42 and 58.90 ± 0.87 , respectively ($P < .0001$)). The decrease in phosphate was significantly higher in Embrace™ varnish than in Profluoride® (mean \pm SD = 29.43 ± 3.98 and 35.50 ± 0.63 , respectively ($P < .0001$)). The increase in Ca/P ratio was significantly higher in Embrace™ varnish than in Profluoride® (mean \pm SD = 2.25 ± 0.43 and 1.65 ± 0.05 , respectively ($P < .0001$)).

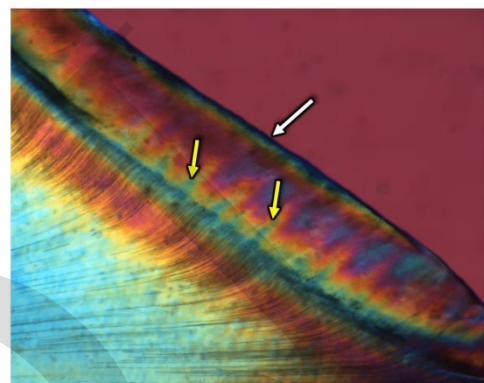


Figure 4: Polarized light photomicrograph of a longitudinal ground section of sound primary enamel showing the prismless enamel layer (white arrow) and alternating Hunter-Schreger bands (Yellow arrows), original Magnification X40.

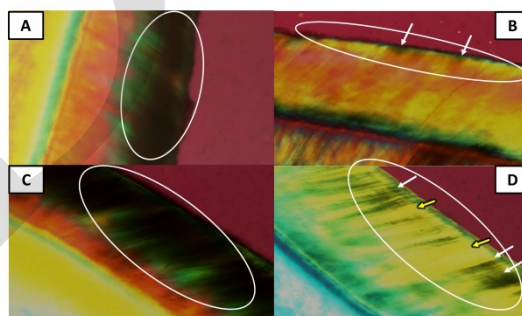


Figure 5: Polarized light photomicrograph of longitudinal ground sections of one specimen of each group showing (A): a specimen of the Embrace™ varnish control group (subgroup IB) showing a broad and deep dark band of demineralization occupying nearly half of enamel thickness and exhibiting strong positive birefringence, (B): a specimen treated with Embrace™ varnish (subgroup IA) showing complete remineralization of the lesion with evident negative birefringence, except for minimal subsurface demineralized areas (Yellow arrows), (C): a specimen of the Profluoride® varnish control group (subgroup IIB) showing a broad, deep dark demineralization band occupying most of enamel thickness, except for a small area adjacent to the amelodentinal junction, (D): a specimen treated with Profluoride® varnish (subgroup IIA) showing areas of regular homogenous remineralization of the lesion (White arrows), while other areas show noticeable reduction in the depth and density of the body of the lesion (Yellow arrows). (Magnification X40)

Table 1: Lesion depth at demineralization, after remineralization and percentage reduction in Embrace™ varnish and Profluoride® varnish.

Group I: Embrace™ varnish			
Lesion depth (µm)	Subgroup IA (Test) (n=12)	Subgroup IB (control) (n=12)	Paired t test (P-value)
Mean±SD	310.75±111.71	644.67±188.93	6.66 (<0.001*)
Group II: Profluoride® Varnish			
Lesion depth (µm)	Subgroup IIA (Test) (n=12)	Subgroup IIB (Control) (n=12)	Paired t test (P-value)
Mean±SD	318.17±176.49	558.58±188.37	7.25 (<0.001*)

Percentage reduction of lesion depth	Group IA (Embrace™ varnish) (n=12)	Group IIA (Profluoride® Varnish) (n=12)	t test (P-value)
Mean±SD	-49.51±17.81	-45.11±20.53	0.55 (0.58)

*Statistically significant at p value ≤0.05
t test: Student's t test

Table 2: Calcium and phosphate enamel content and Ca/P ratio at demineralization and after remineralization in Embrace™ varnish and Profluoride® varnish.

Group I: Embrace™ varnish				
Elemental Content		Subgroup IA (Test) (n=12)	Subgroup IB (Control) (n=12)	Paired t test (P-value)
Ca	Mean±SD	64.80±3.42	57.61±1.34	6.23 (<0.001*)
P	Mean±SD	29.43±3.98	37.53±1.11	6.50 (<0.001*)
Ca/P	Mean±SD	2.25±0.43	1.53±0.07	5.47 (<0.001*)
Group II: Profluoride® Varnish				
Elemental Content		Subgroup IIA (Test) (n=12)	Subgroup IIB (Control) (n=12)	Paired t test (P-value)
Ca	Mean±SD	58.90±0.87	57.60±1.17	4.05 (0.002*)
P	Mean±SD	35.50±0.63	37.52±1.15	7.85 (<0.001*)
Ca/P	Mean±SD	1.65±0.05	1.53±0.07	7.09 (<0.001*)
Elemental content		Group IA (Embrace™ varnish) (n=12)	Group IIA (Profluoride® Varnish) (n=12)	t test (P-value)
Ca	Mean±SD	64.80±3.42	58.90±0.87	5.77 (<0.001*)
P	Mean±SD	29.43±3.98	35.50±0.63	5.12 (<0.001*)
Ca/P	Mean±SD	2.25±0.43	1.65±0.05	4.72 (<0.001*)

*Statistically significant at p value ≤0.05
t test: Student's t test

DISCUSSION

Topical fluoride varnish containing xylitol-coated calcium phosphate (Embrace™ varnish) achieved comparable or significantly superior remineralization results on artificial enamel caries when compared to conventional sodium fluoride varnish (Profluoride® varnish). Hence, the null hypothesis that “there would be no significant difference in the remineralization potential among topical fluoride varnishes when used on primary teeth” was rejected.

Qualitative data obtained from the present study through the polarized light microscope showed that most of the representative lesions treated by Embrace™ varnish exhibited a shift from the positive to negative birefringence with a significant reduction in the mean lesion depth when they were compared to the control. This

may indicates the beginning of the remineralization phenomenon of the enamel lesions. This fact could be due to the presence of calcium, phosphate, xylitol and fluoride ions in the components of Embrace™ varnish.

Profluoride® treated specimens also showed significant reduction in the mean lesion depth when they were compared to the control. The shift from the positive to negative birefringence with reasonable reduction in the depth of the lesion indicating the occurrence of remineralization. These qualitative observations are in consistence with Rirattanapong et al, 2014 (11), who revealed that 5% NaF varnish can promote enamel remineralization.

When comparing the two tested varnishes in the present study, it was found that mean percent reduction of lesion depth in Embrace™ varnish was slightly higher than in Profluoride® varnish, however this difference was not statistically significant. Accordingly, the qualitative and quantitative results obtained are thought to confirm each other. Our findings have implication for the arrest of incipient caries lesions in primary teeth.

The results of the present study conflicted with those of Mohd Said et al, 2017(7), where the latter have observed that the remineralization effect of Embrace™ varnish on enamel was significantly lower than Duraphat varnish (5% NaF). Overall, it was concluded that there was no superior remineralization potential when adding calcium phosphate – based delivery systems to the conventional varnish. It was shown that the combination of xylitol with fluoride restricted the effect of remineralization to the surface enamel only. This is due to the inhibition role of fluoride to the diffusion of xylitol into subsurface enamel layers, therefor reducing its remineralization effect. This theory was however rejected by Cardoso et al, 2016 (26), who compared the effect of varnishes containing xylitol with commercial fluoridated varnishes on the remineralization of artificial enamel caries lesions in situ. Surface hardness and transversal microradiography were used. It was concluded that enamel treated with 20% xylitol plus 5% NaF has a significant remineralization. Xylitol varnishes seem to be favorable replacements for enhancing enamel remineralization.

Milburn et al, 2015(8), compared the quantity and rate of fluoride release from enamel of Embrace™ varnish compared to three other fluoride systems. They concluded that Embrace™ varnish had the highest fluoride release in the first four hours, increasing ten times the release of other fluoride varnishes tested. Conversely, Embrace™ varnish had the highest rate of fluoride depletion of all varnishes tested, so it is beneficial for patients with high caries risk that needs multiple reapplication. This conclusion may explain why Embrace™ varnish in the current study resulted in a non-significant difference in the reduction of lesion depth when compared with Profluoride® varnish. Embrace™ varnish may need multiple application to gain the greatest effect from its components calcium, phosphate, fluoride and xylitol. So, the total outcome results were thought to be time dependent because, it was proposed that the longer the contact of the tooth with tested varnish will be

in the oral cavity, the greater the benefit of remineralization effect and hence the overall results of the Embrace™ varnish. These results were further confirmed by Tulumbaci and Gungormus, 2020 (27), where it was concluded that when Embrace™ varnish was applied on primary teeth which renewed weekly for four weeks resulted in advanced remineralization when compared to Durashield (5% NaF). This is related to the concentration of calcium and phosphate ions which significantly enhance the effectiveness of fluoride in remineralization. Energy dispersive x-ray spectrometer (EDX) was used in the current study for quantitative elemental analysis of the calcium and phosphorus contents in the enamel surface (28). In the present study, Embrace™ varnish treated specimens showed significantly higher mean Ca content and Ca/P ratio than the control. This may be linked to the Ca contained in the Embrace™ varnish. This is in consistence with the polarized light microscope findings obtained in this study which ensures that the remineralization process is dependent on the mineral deposition.

Regarding the Profluoride® varnish, the treated specimens showed significant higher mean Ca content and Ca/P ratio than the control. This finding supports the observations of the polarized light microscope revealing the enhanced enamel remineralization. These results were in accordance with De CarvalhoFilho et al, 2011 (29), revealed that application of fluoride on enamel surface enhancing its stability and remineralization through the deposition of calcium fluoride that changes the chemical component of the enamel hydroxyapatite.

When comparing between both tested varnishes, Embrace™ treated specimens had a significantly higher Ca/P ratio than Profluoride® varnish. Since the two varnishes (Embrace™ and Profluoride®) presented the same fluoride concentration, a probable explanation for the difference between them may be in their elemental mineral content of calcium and phosphate ions which representing better remineralization capacity that confirms the qualitative results of the polarized light microscope by the visualization of heavy areas of mineral gain (high degree of negative birefringence).

The results of the present study are in disagreement with the data presented by Jain et al, 2019 (10), who reported that both the Embrace™ varnish and 5% NaF varnish group were similar in Ca/P and calcium content while the Embrace™ varnish had a higher fluoride content at the end of 4-week remineralization than fluoride varnish. The presence of xylitol in Embrace™ varnish allows for super saturation of calcium and phosphate ions, which in turn allows for greater fluoride uptake. In the presence of saliva, xylitol dissolves to release calcium and phosphate ions. The varnish has an initial high rate of fluoride release which explains the higher fluoride uptake by the demineralized enamel in Embrace™ varnish group (10).

Moreover, Lippert, 2014 (30), stated that the calcium fluoride formed from the applied sodium fluoride varnish is unstable, more soluble and weak at low pH thus, it leaks slowly and easily when exposed to acid. So,

presence of calcium ions as one of the component of the varnish is an important factor to act as an adequate source of minerals for more potential remineralization.

In the present study, Profluoride® treated specimens had a significantly higher mean phosphorus change than Embrace™ varnish. According to Cochrane et al, 2014 (31) and Shen et al, 2016 (32), increased levels of inorganic phosphate may be undesirable for the creation of weakly bound fluoride reservoirs through the reduction of the creation of CaF^+ and CaF_2 and help in the formation of ectopic fluorapatite which decrease the activity of fluoride ion and stimulate calculus development (31, 32). This may give the explanation of the superiority of Embrace™ varnish on Profluoride® varnish among Ca content and Ca/P ratio.

Based on the overall qualitative and quantitative results in the present study, it was suggested that the conjunctional use of calcium, phosphate, fluoride and xylitol will increase the anticariogenic effect, enhance enamel remineralization and give a better demineralization inhibiting effect more than when using fluoride alone.

One of the limitations of the present study was the nonexistence of the natural environment of the oral cavity including the biofilm and oral flora, different salivary components, individuals eating habits and oral hygiene practices. Further studies should be conducted to assess clinically the effectiveness of topical applications of Embrace™ varnish on white spot lesions. Also, the benefits of the present of xylitol in the components of the Embrace™ varnish as an antimicrobial and anticariogenic effects needs to be addressed clinically in future studies.

CONCLUSION

Embrace™ varnish had no significant difference in mean percent reduction in lesion depth than Profluoride® varnish, while it had a better remineralization effect than Profluoride® varnish in terms of higher mineral content.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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