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Remineralization Effect of Two Types of Varnishes on Induced Enamel Caries

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ABSTRACT

Purpose: to assess the remineralization capacity of MI varnish and Bifluorid 10 varnish on induced white spot lesions in deciduous enamel, using Scanning Electron Microscope- Energy Dispersive X-ray. **Materials and methods:** Sixty human caries-free deciduous teeth were collected. The teeth were stored in 0.1% thymol solution. A 4*4*2 window was created on the buccal surface of each tooth using acid-resistant nail varnish. Samples were then divided into 3 groups according to the remineralizing agent used, each group containing 20 teeth. Group I was remineralized with MI varnish, group II was remineralized using Bifluorid 10 varnish, while group III was a control group that didn't receive any remineralization. Then, baseline assessment of Calcium/Phosphorous (% weight) was done using SEM-EDX. Teeth were then immersed in a demineralizing solution at 37°C for 72 hours, until white spot lesions in enamel were visible. SEM-EDX assessment was carried out for the demineralized samples. Remineralization using MI varnish and Bifluorid 10 varnish was done in groups I and II respectively. SEM-EDX assessment was carried out to evaluate the remineralization capacity of each varnish. **Results:** SEM-EDX examination showed that MI varnish had the highest remineralization capacity. The examination showed statistically significant difference between group I and II. They are both superior to the control group. **Conclusion:** Both MI varnish and Bifluorid 10 varnish had acceptable remineralization capacity. MI varnish is preferred.

KEYWORDS

White Spot Lesions, Demineralization, Remineralization, Varnishes.

INTRODUCTION

Dental enamel constitutes the external layer of the tooth crown. Being formed of 92-96% inorganic substance and only 4% organic, enamel is the hardest tissue in the human body. The inorganic part comprises mainly calcium and phosphate, which make hydroxyapatite crystals. These crystals run perpendicular to the DEJ in the form of prisms or rods [(1)].

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Dental caries is the most widely spread chronic infectious disease; it represents a worldwide public health problem, specifically at young age. It mostly starts at a very young age, spreads fast and usually ignored in most cases, leading to major problems related to proper feeding, which generally affects child's performance in all aspects of life[2]. Dental caries has several causes, which means that it cannot be related to a sole cause. There are three primary causes that are considered essential for dental caries to start, which are bacterial biofilm present on tooth surface that later form dental plaque, dietary carbohydrates and the presence of a tooth that can be affected by such influences. Beside these causes, there are other conditions that promote the progression of caries process such as oral hygiene measures, wrong dietary habits, anatomy and position of the tooth, and salivary flow[3].

Dental caries starts initially as white spot lesions (WSLs). These lesions are often seen at young age, accompanying poor oral hygiene. They are seen as white spots on the surface of enamel that occur due to dissolution of its mineral content, leaving this chalky white appearance on the surface. Hence, WSLs represent the initial stage in dental caries before the cavitation stage [4]. International Caries Detection and Assessment System (ICDAS) index is a worldwide recognized system that depends on eyesight in evaluation of dental caries extent. WSLs represent score 1 and 2 on this index. Score 1 means that the WSLs can only be seen when the tooth surface is dry. Score 2 means that WSLs can be seen in both dry and wet states of the tooth. ICDAS was proved to be highly sensitive, reliable and accurately reflects the stage of enamel caries histologically [5]. WSLs are also strongly correlated with orthodontic appliances and their attachments, due to difficulty in performing oral hygiene measures. It was proved that WSLs take about 6 months to form in normal patients while it takes about only 1 month to form in patients who receive orthodontic treatment[6].

WSLs are reversible. At normal pH, saliva is highly saturated with calcium and phosphate ions which are essential for remineralization. It also carries other ions that are of prime importance in remineralization as fluoride. However, fluoride therapy (FT) alone cannot be enough for remineralization to take place. Therefore FT can be enhanced by application of fluoride varnish (FV) or Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP). These components were found to be more efficient in the remineralization process [7].

FT is available in many forms, fluoride toothpastes, fluoride mouthwashes and fluoride varnishes [8]. It was found that application of topical fluoride varnishes no less than two times annually (2-4 times annually) on deciduous teeth reduced the prevalence of caries by 25-30% and the S. mutans count in dental plaque remarkably[9]. It was also found that fluoride found in high concentrations in varnishes cling to hydroxyapatite crystals in enamel forming calcium fluoride. Calcium fluoride acts as a supply for fluoride during high acid concentration state of the oral cavity. This fluoride is then released, hindering the demineralization process, hence, decreasing the formation of WSLs. There are many available formulas for fluoride containing varnishes such as sodium fluoride varnsih with added calcium fluoride (Bifluorid 10 varnish, Voco GmBh), fluoride varnish with added amorphous calcium phosphate (Enamel Pro^{R} Varnish), Casein Phosphopeptide-Amorphous Calcium Phosphate varnish (MI Varnish^{TM} with RECALDENT) and Tri-calcium Phosphate (Clinpro White varnish^{TM})[10]. According to the above mentioned information, this study was conducted to evaluate the remineralizing) capacity of MI varnish (sodium fluoride varnish with added CCP-ACP) and Bifluorid 10 varnish (sodium fluoride varnish with added calcium fluoride), when applied to artificial white spot lesions on tooth enamel.

MATERIAL AND METHODS

Sixty human freshly extracted deciduous teeth were collected from the pediatric dentistry clinic of the Faculty of Dental Medicine for Girls, Al-Azhar University. Teeth were carefully examined for any

defects such as caries, WSLs, fractures, cracks or any other defects. Ethical approval for the use of human extracted teeth was obtained in accordance with the guidelines of the Ethics Committee of the Faculty of Dental Medicine for Girls, Al-Azhar University (REC-PE- 21-09).

Sample size calculation: The calculation was estimated using CDC Epi Info program version 7.2.0.1 (Atlanta, USA) assuming a power of 80% and alpha=0.05 to detect significant difference in the remineralizing capacity of two different fluoride varnishes when applied on artificial white spot lesions on tooth enamel in comparison to control group. A total sample of 60 specimens (20 each group) is needed based on an estimated mean of remineralization of 266.85±37.80 in CPP-ACP varnish specimens compared to 274.83±13.62 in 5% sodium fluoride and 5% calcium fluoride varnish specimens.

First, teeth were cleaned from soft tissues debris using a brush under deionized water. Any tooth with any of the above mentioned defects was excluded from the study [11]. Teeth were stored in 0.1% thymol solution until the study had begun [12].

Samples were prepared first by separating the crown and root parts at the cemento-enamel junction (CEJ) using a diamond disc mounted on a low speed straight handpiece, to get rid of the root part (Fig. 1) [11]. Each sample was then embedded in an epoxy resin block for easy handling, leaving only the buccal surface of the sample exposed [12].

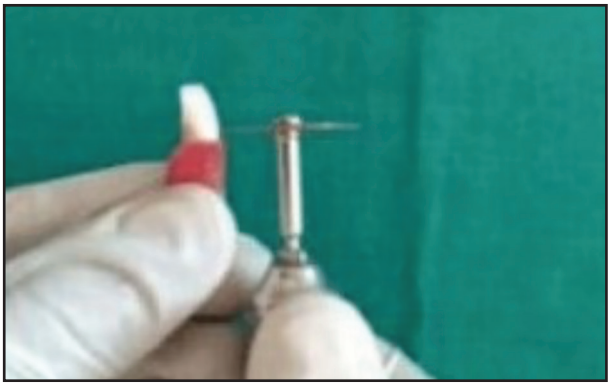

Figure (1) Sample preparation by separation of the root at the CEJ using a diamond disc.

A 4*4*2 mm window was made on the exposed buccal surface of each sample using acid- resistant nail varnish (Fig. 2). Baseline scanning was made for each sample using SEM before demineralization and elemental measurements to assess Ca-P % by weight using EDX. Samples were divided into 3 groups, 20 in each group according to the treatment used for the samples [11].

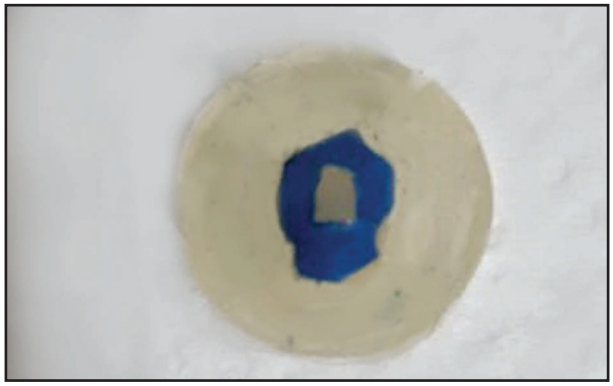

Figure (2) A 4*4*2 mm window created on the buccal surface using acid-resistant nail varnish.

Group I: treated with MI Varnish® (GC Corporation; Tokyo, Japan) (Fig. 3) [14] containing casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) with 5% sodium fluoride [15].

Figure (3) MI varnish (GC, Tokyo, Japan).

Group II: treated with Bifluoride 10 varnish (Bifluorid 10, VOCO, Germany) containing 5% sodium fluoride & 5% calcium fluoride (Fig. 4) [16].

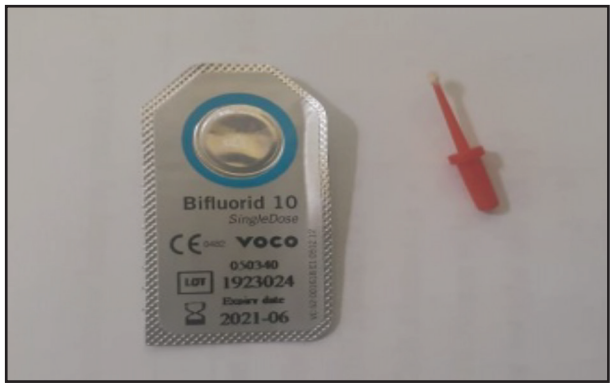

Figure (4) Bifluorid 10 Varnish (Voco, GmbH Cuxhaven, Germany).

Group III: did not receive any treatment [11].

Samples were placed in a demineralizing solution composed of 2.2 mm CaCl2, 2.2 mm NaH2 PO4, 0.05 M lactic acid, and 0.2 ppm fluoride. The pH was adjusted to 4.5 with 50% NaOH. The solution was kept at 37°C. Each tooth was placed individually in a clean container containing 12 ml of the solution and incubated at 37°C for 72 hours, until WSLs were formed [13]. Samples were then rinsed using deionized water to remove any residues of the demineralizing solution. SEM-EDX was made to assess the loss of minerals from enamel after demineralization [11].

Varnishes were smeared on the enamel surface of samples of MI and BIF groups, using micro brushes that come with each varnish. The samples were then left to dry for 2 minutes [17]. Each sample in the 3 groups was then placed individually in a container of 5 ml of artificial saliva at 37°C for 2 weeks, to simulate the treatment in the oral cavity. Artificial saliva is formed of 50 ml NaCl, 0.5 ml CaCl2 and 0.5 ml Na2HPO4, pH 7.0 [15].

Following 2 weeks in remineralization stage, SEM-EDX assessment was made for all 60 samples in the 3 groups. All the samples were assessed for mineral content (mass/atomic percentage) by energy dispersive x-ray analysis (EDX). The digital results of EDX were converted into numerical values of Ca/P % at baseline, after demineralization and remineralization [11].

RESULTS

a) Energy-dispersive X-ray Stereoscopy (EDX) Measurements:

Table (1) *Comparison between the three studied groups regarding percent change of calcium throughout the stages of the study*

Stages	Groups	Mean	SD	SE	95% CI for mean		Min.	Max.	Test value	P-value	Sig.
					Lower	Upper					
From preoperative to demineralization	I	-24.56	13.85	3.10	-31.0426	-18.0811	-44.98	-4.49	0.334	0.718	NS
	II	-18.22	10.93	2.44	-23.335	-13.106	-35.67	-4.16			
	III	-28.61	17.45	3.90	-36.777	-20.442	-46.40	-9.05			
From demineralization to postoperative	I	20.77	8.73	2.85	14.8146	26.7324	11.2	48.22	4.840	0.011	S
	II	5.15	3.78	0.85	3.376	6.914	0.13	11.24			
	III	-3.05	41.53	9.29	-16.394	22.484	-36.05	86.33			
From preoperative to postoperative	I	-9.86	13.45	3.01	-16.1520	-3.5623	-34.09	10.57	7.044	0.002	HS
	II	-14.32	9.38	2.10	-18.703	-9.926	-28.44	-4.03			
	III	-28.58	23.36	5.22	-39.507	-17.644	-65.72	3.21			

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)

•: One Way ANOVA test

The previous table shows that calcium was decreased in the three studied groups from preoperative to demineralization with no statistically significant difference between them regarding calcium percentage of change with p-value= 0.718; also the calcium was increased from demineralization to post-operative in groups I and II and decreased in control group but with statistically significant difference between the three studied groups with p-value = 0.011. The table also shows that the calcium level was decreased in the three studied groups from preoperative to postoperative but decreased in the control group more than the two other groups with p-value = 0.021.

Table (2): *Comparison between the three studied groups regarding percent change of phosphorus throughout the stages of the study*

Stages	Groups	Mean	SD	SE	95% CI for mean		Min.	Max.	Test value	P-value	Sig.
					Lower	Upper					
From preoperative to demineralization	I	-39.53	26.55	5.94	-51.961	-27.107	-79.41	-4.19	1.204	0.307	NS
	II	-28.52	19.11	4.27	-37.463	-19.575	-68.99	-13.35			
	III	-33.58	21.05	4.71	-43.435	-23.73	-63.28	-11.63			
From demineralization to postoperative	I	7.24	4.17	.93341	5.2909	9.1981	4.32	16.53	5.005	0.010	S
	II	40.80	35.58	7.96	24.147	57.449	10.53	116.28			
	III	13.56	50.27	11.24	-9.965	37.093	-26.63	127.17			
From preoperative to postoperative	I	-5.99	7.94	1.77506	-9.7007	-2.2703	-20.93	4.45	5.453	0.007	HS
	II	-5.76	12.30	2.75	-11.514	0.003	-32.93	7.21			
	III	-24.93	33.43	7.47	-40.569	-9.28	-73.06	32.32			

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS).

•One Way ANOVA test

The previous table shows that phosphorus was decreased in the three studied groups from preoperative to demineralization with no statistically significant difference between them regarding phosphorus percentage of change with p-value = 0.307; also the phosphorus was increased from demineralization to post-operative in all the studied groups, but it was significantly higher in groups I and II than control group with p-value = 0.042. The table also shows that the phosphorus level was decreased in the three studied groups from preoperative to postoperative but decreased in the control group more than the two other groups with p-value = 0.044.

b. Environmental Scanning Electron Microscope Evaluation:

Before demineralization in all 3 groups: ESEM image of normal enamel surface (control). It shows enamel perikymata (blue arrows) and enamel rods.

Demineralization in all 3 groups: images were taken after 72 hours of immersion in demineralizing agent. Images showed alteration in the integrity of enamel surface, disturbance in prismatic structure (pores showed by red arrows) in the form of fish scales or honeycomb prismatic pattern destruction.

Remineralization: ESEM image of the control group shows no change from demineralization. MI and BIF groups show enamel surface has somewhat regained its surface properties. Porosity was partially decreased, indicating remineralization. Some of the enamel prism cores have been obliterated.

Group I, (fig. 5):

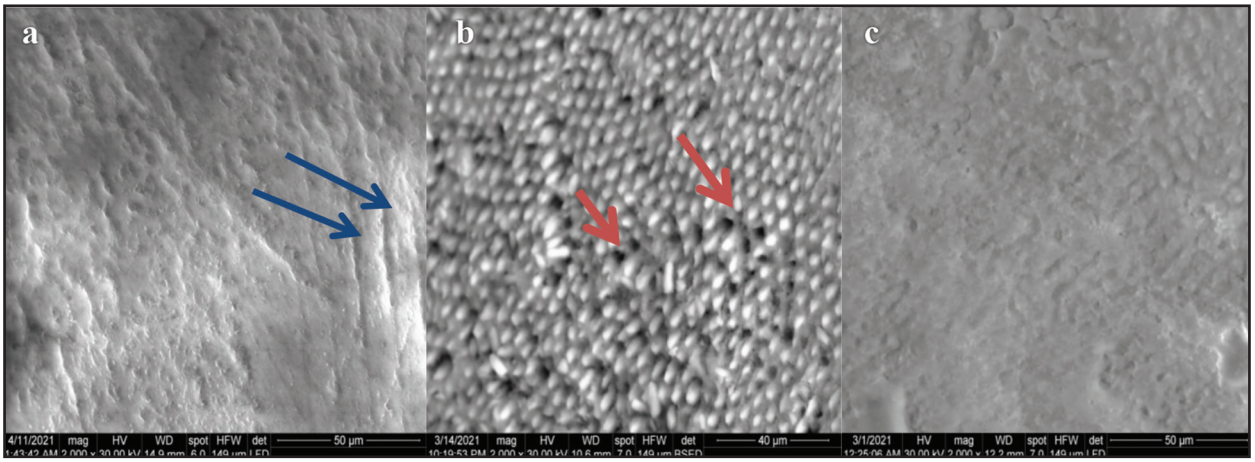

Figure (5) ESEM image (2000x) for the enamel surface of the same sample throughout the 3 stages of the study (a. before demineralization, b. after demineralization, c. after remineralization).

Group II, (fig. 6):

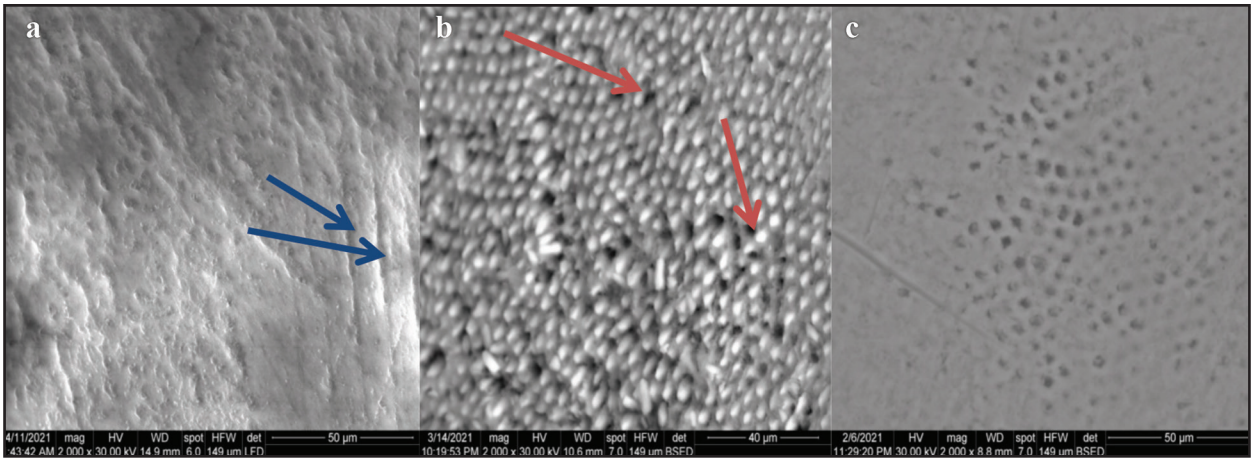

Figure (6) ESEM image (2000x) for the enamel surface of the same sample throughout the 3 stages of the study (a. before demineralization, b. after demineralization, c. after remineralization).

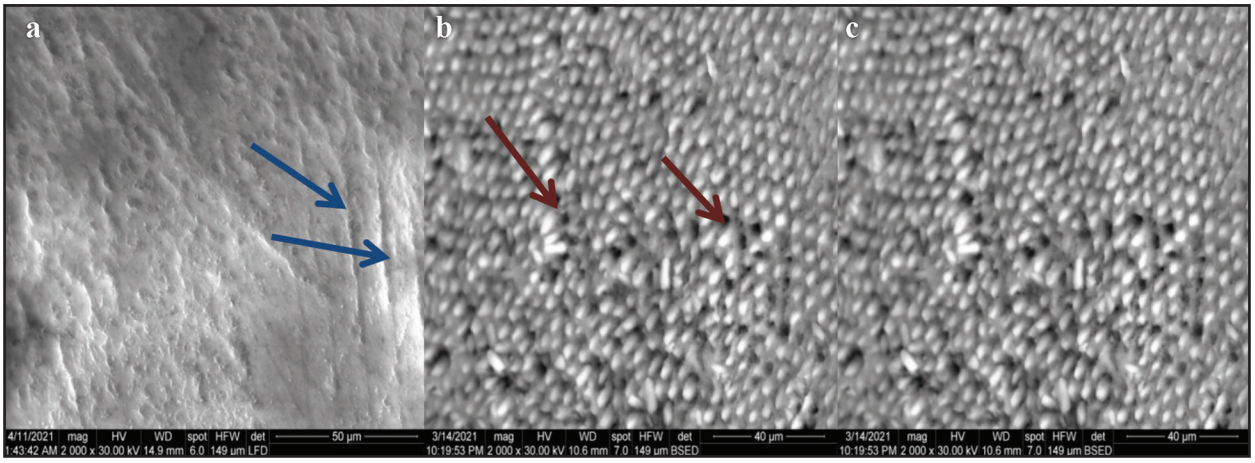

Figure (7) ESEM image (2000x) for the enamel surface of the same sample throughout the 3 stages of the study (a. before demineralization, b. after demineralization, c. no change, as no varnish was applied).

DISCUSSION

The preservation of the demineralization-remineralization equilibrium is the secret to protection against caries incidence. Not long ago, the traditional management of dental caries was to remove the caries and restore it by a filling material. But recently, following years of studies, attention was paid to early diagnosis of dental caries and subsequently treating it in a conservative manner. Trials have been made to shift the caries process from demineralization to remineralization state using varnishes, and they turned out to be successful. Therefore, remineralization serves as a tool in prevention of more aggressive treatment modalities provided that lesions are early explored and the demineralization is still reversible[11].

Deciduous teeth have been used in this study because they are generally weaker than permanent ones in terms of enamel bulkiness and its mineral content. Hence, deciduous teeth have inferior mechanical properties to their permanent successors [17]. Unlike this study, a previous remineralization study was conducted on permanent premolars to assess the remineralization capacity Bifluorid 10 and MI varnish on white spot lesions that are formed around orthodontic brackets [18].

In vitro study models have been widely employed in the evaluation of the remineralization capacity of different materials [17]. However, this in vitro demineralization-remineralization model is different to a large extent in comparison to in vivo studies carried out inside the patient's mouth [11]. This may be attributed to the presence of remineralization inhibitors in saliva, such as statherin, histatins and acidic proline-rich proteins (PRP), which sometimes cause inhibition of varnishes action in in vivo models, by formation of protein-calcium complexes that hinder apatite crystals growth [19]. A previous randomized controlled trial was carried out to assess the remineralization capacity of MI varnish and proved its efficacy in remineralization when evaluated using DIAGOdent [20].

This study compared MI varnish; 5% NaF varnish containing 2% Recaldent™ (CPP-ACP) (GC America Inc., IL) to Voco Bifluorid 10 Varnish (5% calcium fluoride varnish + 5% sodium fluoride VOCO, Germany), to a control group with no varnish used. The comparison was in terms of the remineralization capacity of the two varnishes on induced white spot lesions in deciduous enamel versus no varnish use. The results of this study showed that Ca and P levels decreased initially in the three groups. After treatment with the varnishes and preservation in artificial saliva, Ca and P levels increased in both study groups but remained reduced in the control group. However, Ca and P levels in the MI varnish group were significantly higher than Biflourid 10 group.

Some studies showed similar results to this study in terms of mineral content. A previous study was made that proved that MI varnish showed statistically significant difference in Ca and P content compared to Prevident varnish (composed from 5% sodium fluoride) with p value < 0.0001. The study used the same method of measurement as this study, which is SEM to assess changes in enamel composition and EDX to assess the mineral content [21].

Since both mineral content and surface microhardness affect the surface properties of enamel, the effect of the 2 tested varnishes on both parameters was expected to be comparable. However, a previous study that tested the effect of the two varnishes on surface microhardness using the same demineralization-remineralization protocol showed no statistically significant difference between MI varnish and Bifluorid 10, regarding surface microhardness values. The study used Vicker's hardness test for assessment. The results of this study were different from these of this study, which were obtained by EDX to assess the mineral content. This may be attributed to different means of assessment [22].

Scanning of the enamel surface of the specimens using SEM at baseline showed the sound enamel samples showed smooth surface with well-packed

crystalline rods relatively close and almost parallel with minimal interprismatic matrix area. On demineralization of the specimens of the three groups, SEM images showed apparently intact surface with large focal holes and prismatic pattern of destruction. After remineralization, ESEM images of both MI varnish and Bifluorid 10 varnish groups showed smoother surface, less microporosity due to formation of prism cores. Some of the prisms were completely obliterated. The images of both groups showed no clear difference from each other. ESEM images of the control group showed the same features of the demineralized enamel after immersion in artificial saliva.

Baseline scanning of the specimens of all groups showed similar results to a previous study that described the SEM appearance of normal enamel surface [23]. The features of demineralized enamel in all groups were also similar to several previous studies [24,25].

The features of remineralized enamel in both groups I and II were similar to a previous study that described the remineralization potential of MI varnish [11]. However a previous study that assessed the remineralization potential of Bifluorid 10 varnish showed different SEM features after remineralization, by the formation of a protective pellicle on the surface of the demineralized enamel. This may be due to the different technique of demineralization used in this study, using 37% phosphoric acid for 60 seconds [26].

CONCLUSION

Under limitations of this study, it was concluded that both MI varnish and Bifluorid 10 varnish had an acceptable remineralization capacity, but MI varnish is preferred.

RECOMMENDATIONS

Further studies should be done using in vivo model.

CONFLICT OF INTEREST

There are no conflicts of interest.

FUNDING

No funding was received for this study.

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