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Effect of Different Fluoride Varnishes and Resin Infiltrating Material on the Microhardness of Demineralized Enamel

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Abstract
Purpose: *The purpose of this in-vitro study was to evaluate the effectiveness of two types of fluoride varnishes and a resin infiltrant material on microhardness of induced demineralized enamel surface.*
Materials and methods: *Thirty extracted bovine incisors were selected in this study. The incisal third of the crowns were sectioned by a diamond disc to be used in this study. The labial surface of each sample was tested by knoop hardness test to investigate enamel surface microhardness. The center of the middle third of each sample was covered with an adhesive tape with dimensions 3x3mm.*
The enamel surface of each specimen was protected with 2 layers of nail varnish except the area covered with the adhesive tape. Then the adhesive tape was removed creating a window of sound labial enamel. All samples were demineralized by immersion in 50 ml 0.001M Citric acid solution for 10 minutes and then retested with knoop hardness test.
The demineralized samples were divided into three equal groups I, II and III (10 samples each). Samples in group I were treated with Pro Fluoride varnish, those of group II with MI varnish, while those of group III were treated with Icon resin infiltrant.
All the samples were retested with knoop hardness test to evaluate the changes in enamel surface microhardness.
Results: *Demineralized samples showed decrease of enamel surface microhardness mean value (280.50HK) compared to those recorded for the control samples (366.70HK) and a statistical significant difference (P<0.001) was revealed between them. After remineralization, the effect of the three tested materials on microhardness of enamel was compared and revealed a statistical significant difference (P<0.001) between mean surface microhardness value recorded in group I (411.10HK) versus that recorded in group II (448.70HK). Similar significant difference (P<0.001) was found between mean surface microhardness value recorded in group II (448.70HK) versus that recorded in group III (464.20HK).*
Finally, a statistical significant difference (P<0.001) showed between mean surface microhardness value recorded in group I (411.10HK) versus that recorded in group III (464.20HK) denoting a significant improvement of enamel surface microhardness using Icon compared to profluoride varnish. These results mean that the maximum microhardness was found in group III (Icon) followed by group II (MI varnish) followed by group I (profluoride varnish) and there was significant difference between them p<0.001.
Conclusion: *It was concluded that the maximum microhardness was found in (Icon) followed by MI Varnish followed by profluorde varnish.*
Keywords: *Bovine, Casein phosphopeptide Amorphous calcium phosphate, Demineralization,Enamel, fluoride, Remineralization.*

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I. Introduction

Within the last few years, numerous varnishes with similar sodium fluoride concentrations (5%), but with multiple variations in composition, have emerged, to improve properties like handling, appearance, flavor and in some cases the potentially active ingredients (e.g., tri-calcium phosphate, amorphous calcium phosphate, calcium sodium phosphosilicate and xylitol)[1]leading to additional preventive benefits. Differences in fluoride release patterns can potentially enhance or diminish the efficacy and safety of a varnish.

According to Øgaard[2]fluoride not only increases the rate of remineralization, but can be precipitated with calcium and phosphate ions onto the enamel surface.

Revolutionary approach for treatment of demineralized enamel was represented by a resin infiltrating material (Icon**)**. This breakthrough micro-invasive technology fills and reinforces demineralized enamel.

The Knoop hardness test method, also referred to as a microhardness test method, is used to measure the hardness of teeth and the effectiveness of the remineralization on the microhardness of enamel. The hypothesis of this research is to use Knoop hardness test to evaluate the effect of two different fluoride varnishes and resin infiltrating material on the microhardness of dental enamel.

II. Materials and Methods

The materials which were used in this study are (two types of fluoride varnishes and a resin infiltrating material. The chemical composition, manufacture and web sites are listed in Table 1

Materials	Composition	Manufactures	Web sites
Pro Fluoride varnish (Fig.1)	Pro Fluoride varnish is a colophony-based varnish containing 5% sodium fluoride(22,600 ppm fluoride)	VOCO GERMANY	www.voco.com
MI varnish (Fig.2)	5% sodium fluoride varnish,also contain casein phosphopeptide-amorphous calcium phosphate (CPP-ACP)	GC CORPORATION	www.gcamerica.com
Icon resin infiltrant (Fig.3)	Icon-Etch: Hydrochloric acid, pyrogenic silicic acid, surface-active substances Icon-Dry (99% ethanol) Icon-Infiltrant (methacrylate-based resin matrix, initiators, additives)	DMG – Hamburg, Germany	www.dmg-dental.com
Citric acid	• O 58.29% • H 4.20% • C 37.51%	Food chem. International corporation, Egypt.	
Artificial saliva	• Nacl 0.381 g • Kcl 1.114 g • Cacl2 0.231 g • Deionized water 1000 ml • NaN3 2.2 g • Gastro mucin 2.2 g • KH2po4 0.738 g	Prepared in the department of analytical chemistry, Faculty of pharmacy , Tanta university, Egypt.	

Thirty freshly extracted bovine upper incisors free of cracks were selected from slaughterhouse. The selected teeth were scaled using hand scaler to remove remaining tissue debris. Then they were washed using copious amount of distilled water and stored refrigerated in distilled water to be used within month after extraction.

For each tooth the incisal third of the crowns was sectioned by a slow speed diamond disc with cooling system then stored in a distilled water. All the enamel of the labial surface was protected with 2 layers of transparent acid resistant nail varnish except the area covered with the adhesive tape. Then the adhesive tape was removed from each sample creating a window of sound labial enamel with dimensions (3x3mm).

Knoop hardness test was performed on the labial surface of the samples. The examined samples were stored in artificial saliva in an incubator at temperature 37°C throughout the succeeding steps of the study and it was rechanged daily.

All the samples were demineralized by immersion in 50 ml (0.001M) Citric acid solution for 10 minutes at room temperature then carefully rinsed with distilled water to remove any residual acid on the surface and dried by an intermittent oil free air spray. Each sample examined once more using knoop hardness test to investigate surface hardness of the demineralized enamel surface.

The demineralized samples were then divided randomly into three equal groups (10 samples each), based on the used type of remineralizingagent. **(table 2)**

Table 2: The division of groups

Groups	remineralization regimens
Group I (10 samples)	Remineralized by VOCO Pro Fluoride varnish
Group II (10 samples)	Remineralized by MI varnish
Group III (10 samples)	Remineralized by Icon resin infiltrant

Excessive moisture from the area to be treated was eliminated. The remineralizingagents were applied following the manufacturer's instructions.

Samples of each group were reexamined by knoop hardness test to investigate surface microhardness of the remineralized enamel surface

All data were collected, tabulated and statistically analyzed as dependent data using SPSS version 20. One-way ANOVA test used to compare all the steps of each group. Also T test was used to compare all tested groups together to accept or reject the research hypothesis.

III. Result

Regarding group, I: The knoop surface Microhardness values of Demineralized samples showed The lowest mean value of enamel surface microhardness (280.50HK) while remineralized samples using profluoride varnish recorded the highest mean value (411.10HK) denoting increased surface microhardness. However, the control samples mean value (366.70HK) was recorded as illustrated in table (3) & figure (17&18).

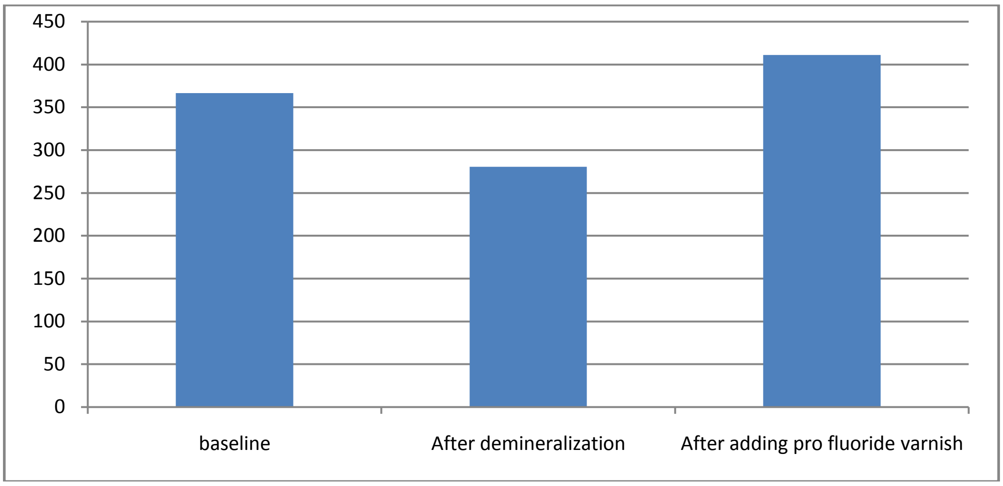
Tukey's test revealed a statistical significant difference ($p<0.001$) between untreated versus both demineralized and remineralized samples as well as the same statistical significant difference revealed between demineralized versus remineralized samples as shown in table (3).

Table (3): Comparison between base line, after demineralization and after remineralization by adding Pro Fluoride varnish according to knoop hardness value (n=10)

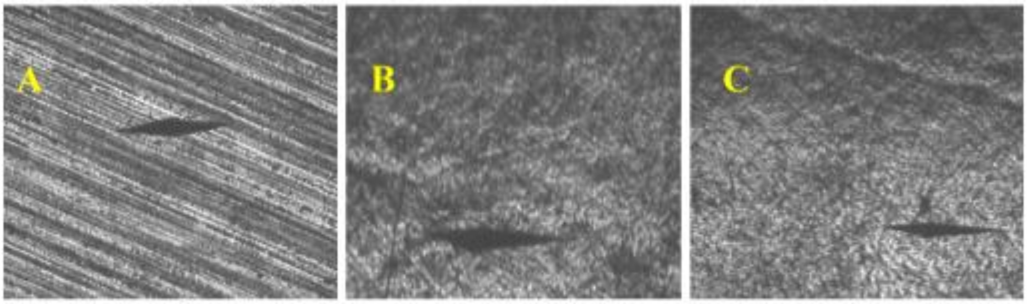
Knop hardness value	baseline samples	After demineralization	After remineralization by adding Pro Fluoride varnish	p
Min. – Max.	359.0 – 372.0	271.0 – 291.0	400.0 – 420.0	$<0.001^{*}$
Mean ± SD.	366.70 ± 4.03	280.50 ± 7.69	411.10 ± 6.76	
Median	367.50	280.0	412.0	

p: value for ANOVA test, sig. bet. groups was done using tukey'stest.

**: Statistically significant at $p \leq 0.05$.*



(Fig. 17): Bar chart representing the Mean Enamel surface microhardness values of baseline data demineralized and remineralized samples after adding Pro Fluoride varnish.



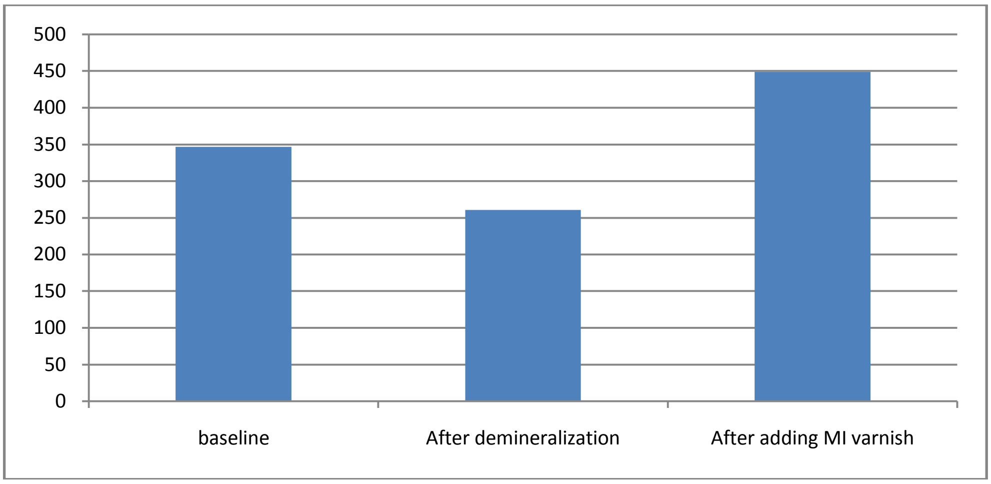
(Fig. 18): Images of knoopmicrohardness test indents of control (A), demineralized (B), remineralized sample by profluoride varnish(C)

In group IITukey's test revealed a statistical significant differences ($p<0.001$) between untreated (346.60HK), the demineralized (260.60HK) and the remineralized samples using MI varnish (448.70HK) and also the same significance was present between demineralized versus remineralized samples as illustrated in table (4) & figure (19&20)

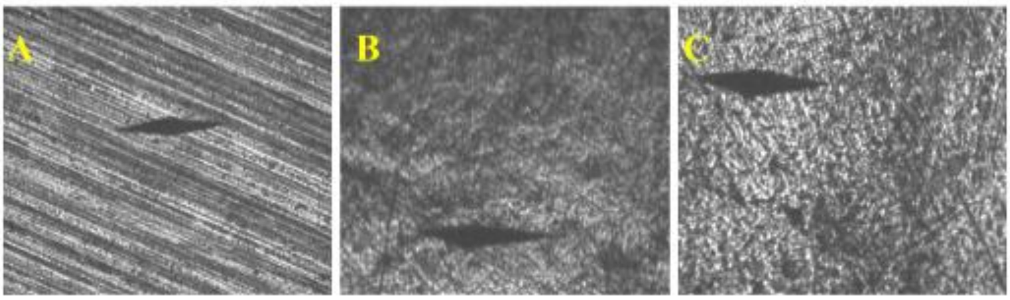
Table (4): Comparison between base line, after demineralization and after adding MI varnish according to knoop hardness value (n=10)

Knop hardness value	baseline samples	After demineralization	After remineralization by adding MI varnish	p
Min. – Max.	338.0 – 357.0	252.0 – 269.0	435.0 – 460.0	<0.001*
Mean ± SD.	346.60 ± 6.83	260.60 ± 6.65	448.70 ± 9.42	
Median	346.50	261.50	449.50	

p: value for ANOVA test, sig. bet. groups was done using tukey's test
**: Statistically significant at $p \le 0.05$.*



(Fig. 19): Bar chart representing the Mean Enamel surface microhardness values of baseline data demineralized and remineralized samples after adding MI varnish.



(Fig. 20): Images of knoopmicrohardness test indents of control (A), demineralized (B), remineralized sample by MI varnish(C)

In group III Tukey's test revealed a statistical significant differences (p<0.001) between untreated (329.80HK) the demineralized (245.50HK) and the remineralized samples using Icon (464.20HK) and also the same significance was present between demineralized versus remineralized samples as illustrated in table (5) & figure (21&22).

Table (5): Comparison between base line, after demineralization and after adding Icon resin infiltration according to knoop hardness value (n=10)

Knop hardness value	baseline samples	After demineralization	After remineralization by adding Icon resin infiltration	p
Min. – Max.	320.0 – 337.0	240.0 – 251.0	455.0 – 470.0	<0.001*
Mean ± SD.	329.80 ± 5.75	245.50 ± 3.63	464.20 ± 5.59	
Median	330.50	245.50	466.0	

p: value for ANOVA test, sig. bet. groups was done using tukey's test
**: Statistically significant at p ≤ 0.05.*

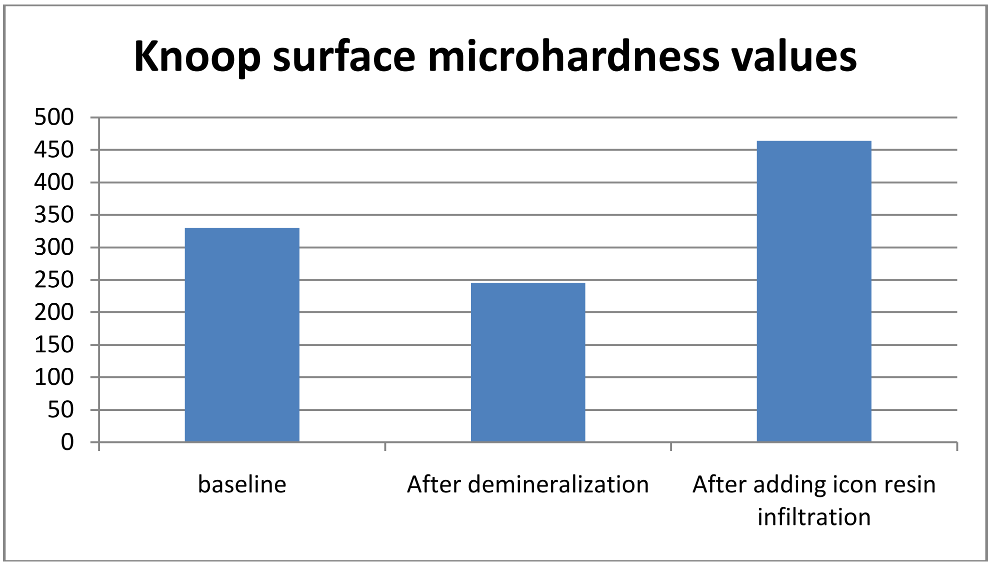
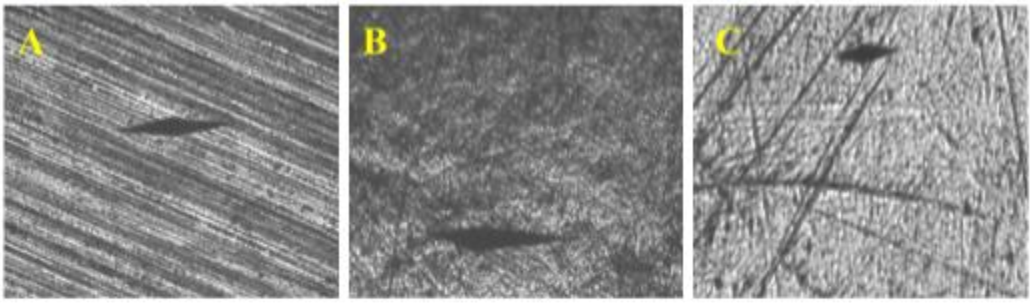


Fig.(21): Bar chart representing the Mean Enamel surface microhardness values of baseline data, demineralized and remineralized samples After adding Icon resin infiltration.

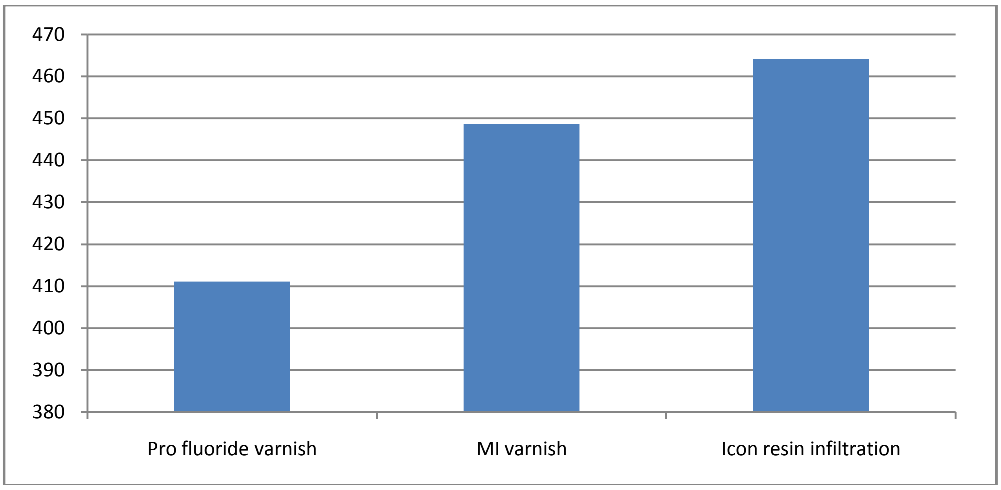


(Fig. 22): Images of knoopmicrohardness test indents of control(A), demineralized(B) ,remineralized sample by Icon(C)

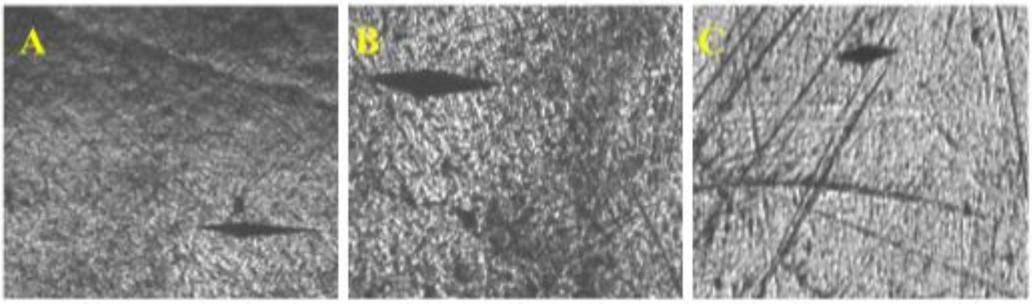
The effect of the tested materials on microhardness of enamel was compared using ANOVA test and Tukey's test which revealed a statistical significant difference (P<0.001) between mean surface microhardness value recorded in group I (411.10) versus that recorded in group II (448.70) denoting a significant improvement of enamel surface microhardness using MI varnish compared to Pro Fluoride varnish, similar significant difference (P<0.001) was found between mean surface microhardness value recorded in group II (448.70) versus that recorded in group III (464.20) which means a significant improvement of enamel surface microhardness using Icon compared to MI varnish,while a statistical significant difference (P<0.001) was present between the mean surface microhardness value recorded in group I (411.10) versus that recorded in group III (464.20) denoting a significant improvement of enamel surface microhardness using Icon compared to Pro Fluoride varnish as illustrated in table (6) & figure (23&24).

Table (6): Comparison between the three studied materials according to knoop hardness value (n=10)

Knop hardness value	Pro Fluoride varnish (n= 10)	MI varnish (n= 10)	Icon resin infiltration (n= 10)	p
Min. – Max.	400.0 – 420.0	435.0 – 460.0	455.0 – 470.0	<0.001*
Mean ± SD.	411.10 ± 6.76	448.70 ± 9.42	464.20 ± 5.59	
Median	412.0	449.50	466.0	



(Fig. 23): Bar chart representing the Mean Enamel surface microhardness values of remineralized samples by the three studied materials.



(Fig. 24): Images of knoopmicrohardness test indents of Samples remineralized by Pro Fluoride varnish(A), MI varnish(B), Icon(C)

It was found from the present study that the maximum microhardness was found in group III (Icon) followed by group II (MI varnish) followed by group I (profluoride) and there was significant difference between them p<0.001.

IV. Discussion

It is important to note that the dental profession's approach to the treatment of caries has been evolving in recent years. A generation ago, it would have been fair to characterize the predominant treatment philosophy as being reactive and focusing on operative intervention. When lesions were detected, and often when they were just suspected, they were restored, and the earlier the better. Over the past several decades, advances in materials and technology and changes in caries epidemiology have all contributed to the emergence of a more proactive, tailored, preventive and conservative treatment philosophy characterized by greater attention to the individual and his or her disease, with reduced emphasis on universal immediate surgical intervention[3]

The selection of this in vitro study was based upon the facts that experimental study can evaluate the effect of a single variable, while keeping all other variables constant. In addition, it is easy, fast and relatively cheap to screen new materials and techniques beside the good correlation that exists between laboratory, and clinical effectiveness.[4] Also another advantage of the in vitro study is the lack of dropout rate, since it does not experience the patients' loss during the follow-up period.

Freshly extracted permanent bovine teeth were used inthecurrentstudyas arepresentative substituteofhuman teeth, following manyauthors[5,6,7]who utilized bovine enamel for studies of demineralization and remineralization reporting that the chemical structure ofbovineenamelas wellasitsresponsetoerosive challenges is comparableto that of humanenamel. Furthermore, bovine teeth have a relatively large flat surface, and are free of carious lesions. [8]Additionally, ethical restrictions and greater access to dental care have made it more difficult to obtain human teeth in good condition for study purposes.

Only the incisal third of the crown was utilized according to Vieira et al.[9] to standardize the samples.

Different storage media as artificial saliva and distilled water were reported for use during testing.[10]Distilled water was currently selected to avoid any chemicals that can be absorbed by, and/ or alter tooth substance.

Artificial saliva was selected as a storage medium simulating the oral environment. This selection was also to overcome the limitations and difficulties of using natural saliva, consuming the time of collection and avoiding quick decomposition. In addition, it will be easy to obtain comparable results with other studies.[11]

Samples were kept in an incubator to resemble the temperature of oral cavity. The storage medium was replaced periodically every 24 hours to minimize deterioration and bacterial growth.[12,13]

Microhardness test is widely utilized to evaluate tooth hardness.[14]Featherstone and Kodaka[15,16] demonstrated the existence of a linear relationship between the square root of Knoopmicrohardness and the mineral content of dental tissues; therefore, the remineralization could be detected by the increase of the enamel microhardness. In the Knoop test method, the indents must be positioned such that there is sufficient clearance from the specimen edge and between the individual indents.

CPP-ACP complexes have been shown to be readily soluble in saliva, [17] creating a diffusion gradient that allows them to localize in supra-gingival plaque.[18]Thus these complexes can enter the lesion as an intact complex or release ions in the plaque fluid to then diffuse into the lesion. It was shown by immuno-localization that CPP were present inside a remineralized enamel subsurface lesion, indicating that they can navigate ions in the lesion.[19]For this reason MI varnish (NAF with cpp-acp) was selected for this study.

After the advent of bonding agents and development of sealants, some studies on infiltration of early carious lesions were conducted.

Icon is an infiltration technique of the demineralizing lesion using light curing resins which was found to be an alternative non fluoride therapeutic approach to prevent the further progression of enamel demineralization. The pores within enamel lesions provide diffusion pathways for acids and dissolved minerals. The aim of infiltration is to occlude these pores, and thus prevent acid penetration into the lesions. Thus Icon resin infiltration was selected for this investigation.[20,21]

Citric acid is a common ingredient in beverages, and, its potential to demineralize dental hard tissue is an increasingly growing health concern for dental personnel. It has been used by many researchers with no consensus regarding the ideal concentration and application time.[22] Therefore, a concentration of 0.001 M Citric acid, a level commonly found in fruit juice drinks was used to induce erosion in this study.[23]

It has been reported that in the initial stage of demineralization when a scaffold of mineral crystals is still present, the lesions could be repaired by remineralization however once the surface was completely lost, demineralization process could not be reversed, for that 10 minutes application time was selected to make sure that this scaffold is not removed.[24]

During the clinical condition any acids may be ingested followed by drinking different amount of water leading to wash the acids containing any ions dissociated from tooth structure, for that in our experimental steps, the samples were rinsed by distilled water after acid application simulating the clinical condition.

On the other hand, rinsing after remineralization removes some softened enamel while keeping the denser interior part of the softened surface relatively intact. For that the samples were rinsed after remineralization procedures.[25]

The current finding where a considerable better enamel surface microhardness values were found after remineralization with (MI varnish) in group II than in group I with Pro Fluoride varnish came in agreement with Reynolds, et al.[26] who attributed that MI varnish, containing fluoride and CPP-ACP was superior to the Pro Fluoride varnish (fluoride-alone varnishes).

El-zankalouny et al.[27] Compared between Icon and MI varnish effect on microhardness of demineralized enamel and found that Resin infiltration significantly increase microhardness of demineralized enamel in comparison to MI varnish, which come in agreement with our results.

The microhardeness results for CPP-ACP group is lower than Icon group and this may be due to the low-viscous resin could penetrate into deeper lesions of the demineralized lesion immediately after treatment. That explains why the microhardeness immediately showed significant recovery for the Icon group

On the other hand, Lippertet al.[28] disagreed with the current results reporting higher percent surface microhardness recovery values of demineralized enamel after application of Pro Fluoride varnish compared to that after application of MI varnish using knoop hardness test.

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Nil.

Conflicts of interest

1. It would be beneficial to perform a clinical studies to confirm these findings.

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