

EFFECT OF THREE TREATMENT MODALITIES FOR WHITE SPOT ENAMEL LESIONS ON ENAMEL SURFACE ROUGHNESS AND MICROHARDNESS

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ABSTRACT

Introduction: White spot lesions (WSLs) are early signs of enamel demineralization, which may or may not lead to the development of caries, this indicates that there is a mineral loss of subsurface area beneath an intact enamel surface. **Aim:** This is a vitro study evaluated the effect of three treatment modalities for white spot enamel lesion namely; resin infiltration, remineralization and micro-abrasion on enamel surface roughness and micro-hardness. **Materials and methods:** Artificial white spot lesions were produced in enamel surface from a sixty intact extracted human permanent premolars were selected for the study. The selected teeth were divided into three groups according to methods of treatment of white spot lesion (20 teeth each); resin infiltration, micro-abrasion and remineralization. For both surface roughness and micro-hardness tests, the assessment was done three times; prior to WSLs productions (baseline) after WSLs production (pretreatment phase) and after treatment (post treatment phase). The different between base line and post treatment phase values will be calculated and represented as the change occurring (value). Statistical analysis was done using one way Anova. **Results:** The results of the current study showed that after treatment of WSLs the surface roughness improved significantly in all tested groups but there was no statistically significant difference between all treated groups. Also, the results of the current study showed that after treatment of the WSLs the microhardness increased in all groups but there was statistically significant difference between Icon[®] treated group compared to both Opalustre[®] and Tooth Mousse[®] treated groups. **Conclusions:** The three tested modalities for the treatment of white spot lesions could improve the surface roughness and the micro-hardness of the treated enamel but the Opalustre[®] and Tooth Mousse[™] were equally effective in improving the microhardness[®] and more effective than Icon.

INTRODUCTION

White spot lesions (WSLs) are early signs of enamel demineralization, which may or may not lead to the development of caries, this indicates that there is a mineral loss of subsurface area beneath an intact enamel surface. This loss of mineralized layer creates porosities that change the refractive index of usually translucent enamel, so the white spot lesions can appear as a milky white opacity when located on smooth surfaces and detected by the naked eye⁽¹⁾.

White spot lesions are not only the result of demineralization, as fluorosis, hypo-mineralization and hypoplasia can also cause lesions. It can also appear after the orthodontics treatment due to plaque accumulation around orthodontics band and brackets. Dental professionals are concerned with performing a differential diagnosis to determine the etiology of white spot lesions, as well as providing appropriate treatment and management the disease⁽²⁾.

The presence of white spot lesions has an effect on the quality of the enamel as the lesions increase enamel roughness and decrease its micro hardness. The surface roughness of the enamel has been closely related to the adhesion ability of bacteria on the surface of the enamel, with the eventual formation of dental plaque, that might lead to white spot lesions progressing into caries⁽³⁾. Furthermore, a decrease in enamel micro-hardness leads to more liability for wearing or abrasion, attrition, erosion, and abfraction. This the tooth surface becomes more sensitive and fragile⁽⁴⁾.

The conventional treatment approach is based on restoration, which, in most instances, is quite invasive. Efforts should be made to achieve an ideal function and esthetics along with preserving the sound tooth structures as much as possible. Since most patients requiring treatment for white spot lesions are adolescents or young adults, minimally invasive treatments are needed to prevent excessive sacrifice of tooth material at an early age.

Caries infiltration is a minimally invasive technique for the management of smooth surface and proximal non-cavitated caries lesions. Several remineralization products have been presented to this end, such as fluoride, casein phosphopeptide, amorphous calcium phosphate, and microabrasion . Low-viscosity light-cured resins are another popular approach. The infiltration of resins creates

a diffusion barrier inside the enamel lesion body ,retarding enamel dissolution , and the retention loss is unlikely to occur⁽⁵⁾.

Several conservative approaches are recommended for treatment of WSLs. These approaches start with remineralization using fluoride therapy, casein–phosphopeptide–amorphous calcium phosphate pastes, Novamin (calcium sodium phosphosilicate) in different forms. And proceed to more invasive approaches including micro-abrasion, conventional bonding and various types of veneers. Recently, a new non-invasive technique for treating white spot lesions by resin infiltration was introduced to the market in 2010, by “DMG,” as a product called “Icon.”⁽⁶⁾

Different studies of non-invasive infiltration technology have been conducted, Yazkan et al,⁽⁷⁾ evaluated the effects of resin infiltration and microabrasion on incipient carious lesions by surface microhardness, roughness and morphological assessments, and resistance to further acid attack of treated lesions. Eighty artificially-induced incipient lesions were randomly divided into five groups (n¼16): resin infiltration with an adhesive resin (Excite F, Ivoclar Vivadent, Schaan, Liechtenstein), resin infiltration with a resin infiltrant (Icon, DMG, Hamburg, Germany), microabrasion without polishing (Opalustre, Ultradent, South Jordan, UT, USA), microabrasion with polishing (Opalustre, Ultradent, Diamond Excel, FGM, Joinville, SC, Brazil), and distilled water (control group). All specimens were exposed to demineralization for another 10 d. Microhardness, roughness and morphological assessments were done at baseline, following initial demineralization, treatment and further demineralization. They concluded that icon infiltration and microabrasion technique appeared to be effective for improving microhardness. Icon appeared to provide reduced roughness, although

not equal to sound enamel. Further research is needed to elucidate their clinical relevance.

Attia and Kamel⁽⁸⁾, determined the effect of fluoride gel, Remin Pro, and GC tooth mousse plus in changing surface roughness of enamel after bleaching procedures. Dental enamel blocks measuring 2 × 3 × 4 mm were prepared from non-erupted human third molars. The 38% hydrogen peroxide in-office bleaching protocols were performed. The specimens were randomly divided into three groups (n = 10 samples per group). according to the remineralizing agent used: group 1: using fluoride gel, group 2: using Remin Pro, and group 3: using GC tooth mousse plus. Measurements of surface roughness

were carried out using a contact stylus profilometer before bleaching, after bleaching, and after remineralization. They concluded that GC tooth mousse plus and Remin Pro are more effective in reducing enamel surface roughness after bleaching than fluoride product.

Many investigations have been made on the effect of resin infiltration, remineralization and micro-abrasion on the enamel surface roughness and micro-hardness. The present investigation is designed to investigate the ability of different modalities of treatment to improve the enamel surface roughness and micro-hardness.

MATERIALS AND METHODS

I. Materials:

Three materials used for treatment of WSLs were selected in this study:

Table (1) Specifications, manufacturers and chemical composition of the materials used in the study:

Materials	Composition	Manufacture	Lot No.
Icon [®]	<ul style="list-style-type: none"> Icon-Etch: 15% Hydrochloric acid, pyrogenic silicic acid, water, additives. Icon-Dry: 99% ethanol Icon-Infiltrant: Methacrylate-based resin matrix, TEGDMA, initiators, additives 	DMG, (Hamburg, Germany)	742285
Tooth Mousse [®]	Casein phosphopeptide-amorphous calcium phosphate.	GC Asia Dental Pte. Ltd	151003s
Opalustre [®]	A viscous 6.6% hydrochloric acid slurry contains silicon carbide microparticles.	Ultra- Dent	
Deminerlizing solution	Citric acid 0.3 mol/L, pH2	Solution prepared at chemistry lab in Suez Canal Uni.	

II-Methods:

This study was included two laboratory tests: surface roughness test and microhardness test.

Specimens selection and grouping:

In this a vitro study, a total number of 60 extracted caries-free human permanent premolars were used. Teeth were cleaned with water to remove any blood and mucous, scaled with periodontal scaler to remove any plaque, calculus and attached periodontal tissues, polished with fine pumice and soft rubber cups at conventional speed. The teeth were examined for freedom of cracks using a magnifying lens. All the teeth exhibiting any signs of caries, micro cracks or any other defective structure were discarded. Teeth were stored in saline solution at room temperature till the time of their use (within one month from the extraction date). This study was not submitted to the Ethics Committee because it was registered in (2016) . This study included 60 specimens divided into five groups: Group A: (Baseline group) were included all 60 sound specimens. Group B, (demineralized group) included the same 60 specimens after immersion in demineralized solution. Then after demineralization 60 demineralized specimens were divided into three equal groups according to the treatment modalities of white spot lesions (20 specimens each): Group C: Resin infiltration by Icon, Group D: Remineralizing agent by Gc tooth mousse and Group E: Micro-abrasion by Opalustre. All groups were subjected to two tests (surface roughness test and micro hardness test) at three phase (prior to WSLs productions (baseline), after WSLs production (demineralization phase) and after treatment (post treatment phase). The selected teeth for the surface roughness test were left intact and no surface preparation was done. For the microhardness test, the selected teeth were sectioned mesiodistally parallel to the long axis of crown and

roots were cut at the level of the cemento enamel junction by using a hard-tissue microtome (Isomet. 1000, Buehler Ltd., Lake Bluff, USA), to produce a relatively flat buccal surface to allow microhardness testing. The enamel slabs (approximately 5x5 mm²) are cemented on thin acrylic resin bases (2cm x 3cm) by using flowable composite (Meta Biomed, Inc., Chungbuk, Korea)

Preparation of White Spot- Like Lesions:

All specimens were designate the test area, enamel surface of each specimen was partly covered with acid-resistant nail varnish, leaving an experimental window of sound enamel of about (2x2 mm), made by cutting the window on sticky paper and stick it on the specimen surface. After application of the nail varnish, the sticky paper was removed. Specimens were subjected to a demineralization protocol for 1 hour until a white spot lesion was developed or (chalky white lesion). They were rinsed using air water spray for 1 minute to ensure washing all remnants. All the prepared specimens were stored in saline at room temperature until being subjected to the assigned treatment modality.

Treatment modalities of specimens:

Each group (n=20) was subjected to one of three treatment protocol according to manufacturer instructions either:

Group C: Resin infiltration by Icon® system:

According to manufacturer's instructions, the resin infiltration Icon® (DMG, Hamburg, Germany) was used:

- ICON-Etch: (DMG, Hamburg, Germany) 15% hydrochloric acid was applied over the artificial WSL by using sponge tip supplied in the ICON kit and fitted it into ICON-Etch syringe, acid gel

was extruded by twisting the syringe and left for 2 minutes then rinsed for 30 sec. with water jet followed by drying with oil-free compressed air for 20 sec.

- **ICON-Dry:** (99% ethanol-drying agent,) supplied in a syringe in the ICON kit, this drying step involves 30 sec. of application of the ICON-Dry followed by compressed oil-free air drying.
- **ICON-Infiltrant:** a resin with a high penetration coefficient, was then applied with the supplied applicator in a series of two applications, first for 3 minutes and then for an additional 1 minute and cured after each application for 40 sec.

Group D: Remineralization by GC-Tooth Mousse®:

GC-Tooth Mousse™ was applied on the artificial WSL by using cotton applicator tips three times daily for three minutes for fourteen days. The specimens were stored in natural saliva in-between the applications. The unstimulated natural saliva was collected from one patient. A patient was sitting in an upright position, with head inclined forward and with minimal and oro-facial movement, asked not to swallow saliva and stay motionless, saliva was collected passively in the floor of the mouth with low forced spitting. Saliva was collected in a plastic cup.

Group E: Micro-abrasion by Opulastre®:

A thin layer of Opulastre® slurry was applied on the artificial WSL by using Opulastre syringe then used a polishing rubber cup (DENTP® Prophlaxis polishing Rubber Cup Latch) in slow PRM hand piece (COXO®, Max 30,000 R.P.M) under medium to heavy pressure for 60 seconds to compress Opalustre® slurry on tooth surface.

Intermittent rinsing and inspection as recommended by manufacturer until disappearance of WSLs was achieved.

Testing phase:

All groups were subjected to two tests (surface roughness test and micro hardness test) at three phase (prior to WSLs productions (baseline), after WSLs production (demineralization phase) and after treatment (post treatment phase).

The surface roughness assessment: The surface roughness measurement was performed qualitatively and quantitatively by using Environmental Scanning Electron Microscope (FEI, Quanta 200, Multinational gathered at Netherlands)

The micro-hardness assessment: The micro-hardness measurements was performed qualitatively and quantitatively by using digital Vickers micro-hardness tester.(Wilson micro- hardness tester model Tukon 1102 Gemany).

Statistical Analysis

Data were analyzed using Microsoft Excel software. Data were then imported into Statistical Package for the Social Sciences (SPSS version 20.0) (Statistical Package for the Social Sciences) software for analysis. According to the type of data qualitative represent as number and percentage, quantitative continues group represent by mean \pm SD, the following tests were used to test differences for significance, difference and association of qualitative variable by Chi square test (X²). Differences between quantitative independent groups by t test, multiple by ANOVA, paired by paired t . P value was set at <0.05 for significant results &<0.001 for high significant result.

RESULTS

Assessment of surface roughness

Table (2) and fig (1) represent the descriptive statistics of surface roughness of all groups expressed in mean and standard deviation in μm . The Surface roughness of the sound enamel of Icon[®] group recorded (168.83 ± 14.68), the tooth Mousse[®] group recorded (176.9 ± 10.6) and the Opalustre[®] group recorded (171.49 ± 15.2) when tested before demineralization. There was no

significant difference between all the sound groups. After demineralization, the Icon[®] group was (218.91 ± 13.75), tooth Mousse[®] group was (226.16 ± 6.2) and the Opalustre[®] group was (234.44 ± 18.6). There was no significant difference between all demineralized groups, Also there wasn't a statistical different between the groups after the application of treatment with Icon[®] group the mean and standard deviation was (188.19 ± 9.61), Tooth Mousse[®] group was (180.6 ± 7.46) and Opalustre[®] group was (182.4 ± 18.2).

Table (2) Descriptive statistics of surface roughness means and standard deviation in (μm):

	Icon [®]		Tooth Mousse		Opalustre [®]		F	P
	Mean	SD	Mean	SD	Mean	SD		
Sound	168.83 ^c	14.68	176.9 ^c	10.67	171.49 ^c	15.2	7.838	0.591
Demineralized	218.91 ^a	13.75	226.16 ^a	6.2	234.44 ^a	18.6	1.576	0.247
Treated	188.19 ^b	9.61	180.6 ^b	7.46	182.4 ^b	18.2	0.492	0.623

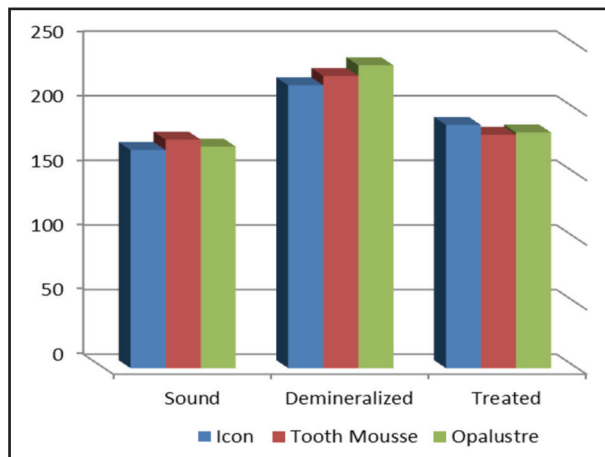


Fig. (1) Column chart represent the phases subjected to surface roughness test

Assessment of Micro-hardness:

Table (3) and Fig (2) represent the descriptive statistics of microhardness of all treated groups with (Icon[®], Opalustre[®] and Tooth Mousse[®]) expressed in means and standard deviation in (VHN). The microhardness of the sound enamel of the group treated with Icon[®] was (364.81 ± 31.2), treated with Tooth Mousse[®] was (344 ± 29.3) and those treated with Opalustre was (356.84 ± 22.64) with no statically significant difference between the three groups. The demineralized enamel showed the lowest microhardness which is not statistically different between the all groups where Icon[®] group was (172.66 ± 12.7), Tooth Mousse[®] group was (188.43 ± 15.41) and Opalustre[®] was (157.32 ± 10.2). After the treatment

Table (3) Descriptive statistics of micro-hardness means and standard deviation in (VHN)

	Icon®		Tooth mousse®		Opalustre®		F	P
	Mean	SD	Mean	SD	Mean	SD		
Sound	364.81 ^a	31.2	344.93 ^a	29.3	356.84 ^a	22.64	0.639	0.545
Demineralized	172.66 ^d	12.7	188.43 ^d	15.41	157.32 ^d	10.2	15.923	0.573
Treated	210.69 ^c	10.39	250.26 ^b	35.3	265.05 ^b	20.03	6.747	0.011*

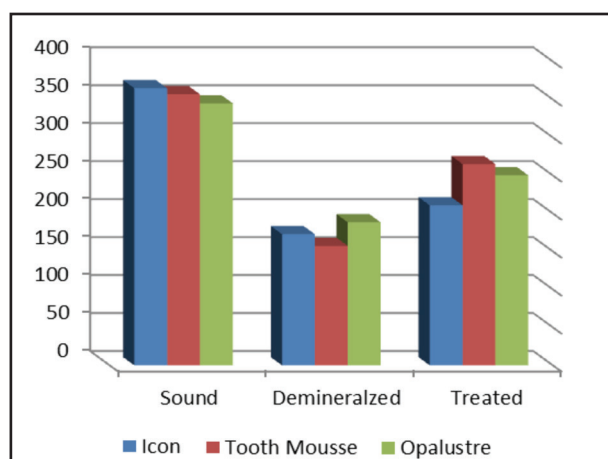


Fig. (2) Column chart represent the phases subjected to micro hardness test.

with the three materials, the micro-hardness of the group treated with Icon® was (210 ±10.39), Tooth Mousse® was (250.26 ±35.03) and Opalustre was (265.05 ±20.03), where there is highly significant difference between Icon group and each one of the others group.

DISCUSSION

This in vitro study was designed to evaluate the effect of three treatment modalities for white spot enamel lesion namely; resin infiltration (Icon®), remineralizing agent (Tooth Mousse®) and micro-abrasion (Opalustre®) on enamel surface roughness and micro-hardness.

Icon® was introduced as a treatment modality for treating early WSLs. It is a low-viscosity light-curing resin that has been optimized for rapid penetration into the porous enamel. Tiny pores within the enamel lesion body act as diffusion pathways for acids and dissolved minerals. An alternative approach to arrest carious lesions is to infiltrate these pores with light-curing resins. This approach not only seals the micro-porosities but also, blocks the access of acids to any remaining pores. The resin infiltrant will mask the opaque white spot lesions in smooth buccal and interproximal surfaces⁽⁷⁾.

The casein phosphopeptide–amorphous calcium phosphate (CPP–ACP) complex is generally marketed in the USA as MI Paste and MI Paste Plus and outside the USA. As CPP–ACP has the ability to localize ACP at the tooth structure, increasing the level of calcium phosphate in plaque and hence act as a calcium phosphate reservoir, buffering the free calcium and phosphate ion activities, thereby helping to maintain a state of supersaturation with respect to tooth enamel thereby it decreases enamel demineralization and enhances enamel remineralization⁽⁸⁾.

The microabrasion technique using Opalustre® (Ultradent Products) is considered effective in the removal of inactive carious white spot lesions that have been pigmented after remineralization to produce regular surface. Opalustre® is a lower concentration of hydrochloric acid (6.6%) and silicon carbide abrasive powder in a silica gel for

rotary application. The technique removes the porous surface enamel layer, as well as the entrapped stains, by rubbing gel that contains an acid and abrasive compound. Opalustre[®] produced enamel loss ranging between 25 and 200 μm , making it acceptable for clinical use⁽⁹⁾.

The results of the current study showed that the baseline of surface roughness was lowest recorded and similar in all tested groups indicating a standard baseline for all groups. After demineralization the surface roughness significantly increased in all groups with no significant difference between the groups, again confirming the standardization of the demineralization protocol used. Also, the results of the current study showed that after treatment of WSLs the surface roughness improved significantly in all tested groups but there was no statistically significant difference between all treated groups. Improvement in surface roughness did not match the sound enamel baseline.

This finding is also in agreement with Soveral *et al.*⁽¹⁰⁾ who found that both sound enamel and WSLs decreased the roughness after resin-infiltration application, and these results have clinical importance. The oral cavity constantly undergoes a dynamic demineralization-remineralization cycle that promotes natural healing processes. On the one hand, oral biofilm and dietary acids can contribute to create porous lesions on enamel, and, on the other hand, saliva, sealants, antibacterials, fluoride, and a controlled diet with less sugar and starchy foods promote a non-demineralizing environment. Hence, infiltrative resins may play a role not only interventional but also preventive in the enamel roughness resulting throughout life.

This result is in partial agreement with Yazkan & Ermis⁽⁷⁾ who compared the effect of resin infiltration and microabrasion on the microhardness, surface roughness and morphology of incipient carious

lesions. They found in their study that infiltration of incipient lesions with resin infiltrant resulted in significantly lower Ra values when compared to the surface roughness after initial demineralization. However, the roughness of infiltrated lesions was still significantly increased if compared to the roughness of sound enamel, but the micro-abrasion of enamel did not provide the same degree of improvement in surface roughness as did the resin infiltration. They explained their results by the fact that Icon can easily penetrate into the enamel subsurface due to its low viscosity and small contact angle, which could thus also explain the thinner resin layer on lesion surfaces measured by 3D topography images in studies.

Also, this finding is in agreement with Attia & Kamel⁽⁸⁾ who determined the effect of fluoride gel, Remin Pro, and GC Tooth Mousse plus in changing surface roughness of enamel after bleaching procedures. They found that GC Tooth Mousse plus and Remin Pro are more effective in reducing enamel surface roughness after bleaching than fluoride product. They attributed their finding to the small size of the CPP ACP nanocomplex.

This result in contrast with Arora, *et al.*⁽¹³⁾ who found that resin infiltration (Icon) showed minimum surface roughness into initial enamel carious lesions. They attributed their finding to the fact that the resin used in this study was triethylene glycol dimethacrylate (TEGDMA.) TEGDMA has been shown to have a relatively high solubility that influences the water absorption and the degradation of the polymer.

Also, a study by Bayram, *et al.*⁽¹⁴⁾ evaluated and compared the effects of remineralizing agents (fluoride varnish and CPP-ACP containing paste), as well as saliva, on stripped enamel surfaces. They found that the surface roughness of stripped enamel surfaces after exposure to saliva and remineralizing agents has lower value than intact enamel. They

attributed this finding to the fact that intact enamel has naturally a rougher surface than stripped enamel due to the presence of the outermost aprismatic enamel surface.

Also, this finding opposed with those of Silvaa, *et al.*,⁽¹⁵⁾ who found that enamel roughness increased after the microabrasion treatment following the initial abrasion, due to different particle size of agents and greater granulation sizes associated with Opalustre's micro abrasive system at 20–160µm.

Also, the results of the current study showed that after treatment of the WSLs the microhardness increased in all groups but there was statistically significant difference between Icon® treated group compared to both Opalustre® and Tooth Mousse® treated groups. Although the three treatment modalities increased the recorded microhardness significantly compared to the demineralized enamel, yet the recorded microhardness after treatment was significantly lower that of sound baseline enamel.

This result in agreement with Zakizade, *et al.*⁽¹⁶⁾ who confirmed that the resin infiltration cannot return the microhardness of WSLs to that of sound enamel, Although resin infiltration might increase the microhardness, the establishment of the polymeric chain does not always happen in the entire lesion. Therefore, the inability of a strong intermolecular bond plus the non-infiltration of the resin in the entire enamel lesion can prevent the full recovery of the enamel microhardness.

Also, this finding is in agreement with Ghazaleh *et al.*,⁽¹⁷⁾ who found all the remineralizing agents were effective for rehardening the enamel after microabrasion. They explained their result by the remineralization effect of CPP-ACP paste involves the incorporation of CPP part of this complex to the enamel and biofilm, in order to deliver the calcium and phosphate ions to the enamel rods and reconstruct the apatite crystals.

Concerning Opalustre, this result is confirmed with Zavala-Alonso *et al.*,⁽¹⁸⁾ who found that Opalustre® increases the microhardness of fluorotic enamel, although it does not recover its baseline microhardness. They attributed their results to that microabrasion with HCl significantly removed the superficial enamel layer. The results of microhardness match those found in the SEM since the images after the placement of each of the microabrasion showed irregular surface, exhibited areas with a selective conditioning etching and others with a non-selective pattern.

On the other hand, this finding is in disagreement with Horuztepe & Baseren,⁽¹⁹⁾ who found that the microhardness of resin infiltration is increased than the microhardness of untreated or remineralized carious lesions enamel surface. They attributed their finding to the ability of the low-viscosity resin to fill the spaces between the remaining crystals of the porous lesions and re-harden the demineralized tissue, thereby improving their mechanical strength.

Also, this result is in disagreement with Simsek, *et al.*,⁽²⁰⁾ who found there were no significant increases in the surface microhardness values of artificial saliva or CPP-ACP. They attributed their findings to CCP-ACP being more effective in deep caries lesions. CCP-ACP promoted the formation of a mineral deposition and complete layer on enamel surface in SEM.

Regarding Opalustre, this result in contrast with Franco *et al.*,⁽²¹⁾ who demonstrated a decrease in enamel microhardness after enamel microabrasion. This significant reduction was justified by the presence of 6.6% hydrochloric acid in the microabrasive compound. This is because the stain removal depends on the erosive action of the acid present in the microabrasive product justifying the reduction in mineralization of the enamel surface after microabrasion.

CONCLUSIONS

Under the conditions of the present study the following conclusions could be derived:

1. The three tested modalities for the treatment of white spot lesions could improve the surface roughness of the treated enamel, yet did not restore the baseline sound enamel smoothness.
2. The three tested modalities for the treatment of white spot lesions could improve the microhardness of the treated enamel, yet did not restore the sound enamel baseline microhardness.
3. Both Opalustre and Tooth Mousse were equally effective in improving the microhardness and more effective than Icon.

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