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ORIGINAL ARTICLE DOI: https://doi.org/10.4322/bds.2022.e3026

Effect of resin infiltration and bleaching on surface roughness and microhardness of human enamel with induced white spot lesions

Efeito do infiltrante resinoso e do clareamento na rugosidade e microdureza de superfície do esmalte humano com lesões de mancha branca induzidas

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ABSTRACT

Objective: To comparatively evaluate the effect of resin infiltration, bleaching and bleaching followed by resin infiltration on the surface roughness and microhardness of human enamel with induced white spot lesions (WSLs) and their resistance to acidic challenge. **Material and Methods:** Sixty human specimens were randomly divided into three groups (n=20) according to the treatment modality applied; group I Icon® resin infiltration, group II bleaching and group III bleaching followed by Icon® resin infiltration. For each treatment modality, 10 specimens were tested for surface roughness and another 10 for microhardness. WSLs were artificially induced in all specimens and after treatment, all specimens were subjected to acidic challenge. Surface roughness was measured by the tapping mode of the atomic force microscope (AFM) and microhardness was measured by digital Vickers hardness tester at baseline, after induction of WSLs, after treatment and after acidic challenge. **Results:** Groups I and III showed significant reduction in surface roughness after treatment, while group II showed significant increase. Groups I and III showed significant increase in the microhardness after treatment, while group II showed insignificant increase. The three tested groups showed significant increase in surface roughness values and significant reduction in microhardness after acidic challenge. **Conclusion:** Resin infiltration and bleaching followed by resin infiltration reduced the surface roughness and enhanced the microhardness of the WSLs. The three treatment modalities failed to resist acidic challenge resulting in increasing surface roughness and reducing microhardness.

KEYWORDS

AFM; Bleaching; Microhardness; Resin infiltration; White spot lesions.

RESUMO

Objetivo: Avaliar comparativamente o efeito do infiltrante resinoso, clareamento e clareamento seguido de infiltração resinosa sobre a rugosidade e microdureza superficial do esmalte humano com lesões de manchas brancas induzidas (WSLs) e sua resistência ao desafio erosivo. **Material e Métodos:** Sessenta espécimes humanos foram divididos aleatoriamente em três grupos (n = 20) de acordo com a modalidade de tratamento aplicada; grupo I infiltrante resinoso Icon®, grupo II clareamento e grupo III clareamento seguido de infiltração resinosa Icon®. Para cada modalidade de tratamento, 10 corpos-de-prova foram testados para rugosidade superficial e outros 10 para microdureza. WSLs foram artificialmente induzidos em todas as amostras e, após o tratamento, todas as amostras foram submetidas ao desafio erosivo. A rugosidade de superfície foi medida por microscopia de força atômica em modo de contato intermitente (AFM) e a microdureza Vickers foi medida inicialmente, após a indução de WSLs, após o tratamento e após o desafio ácido. **Resultados:** Os grupos I e III apresentaram redução significativa da rugosidade superficial após o tratamento, enquanto o grupo II apresentou aumento significativo. Os grupos I e III apresentaram aumento significativo na microdureza após o tratamento, enquanto o grupo II apresentou aumento insignificante. Os três grupos testados mostraram aumento significativo nos valores de rugosidade superficial e redução significativa na microdureza após o desafio erosivo. **Conclusão:** O infiltrante resinoso e o clareamento seguido de infiltração resinosa reduziram a rugosidade de superfície e aumentaram a microdureza dos WSLs. As três modalidades de tratamento falharam em resistir ao desafio erosivo, resultando em aumento da rugosidade de superfície e redução da microdureza.

PALAVRAS-CHAVE

AFM; Clareamento; Microdureza; Infiltrante resinoso; Lesões de mancha branca.

INTRODUCTION

White spot lesions (WSLs) can be carious as that occurring in orthodontic patients or non-carious such as fluorosis and enamel hypoplasia [1]. The occurrence of WSLs in orthodontic patients was reported to be as high as 97%, thus they are considered one of the common complications of orthodontic treatment [2]. This was attributed to improper oral hygiene measures, microbial adhesion, biofilm formation, and the acids produced from the bacteria colonizing this biofilm lead to enamel demineralization [3]. These WSLs usually occur as early as one month after orthodontic appliances fixation and continue for five years after they are removed compromising the esthetics of the patients and self-esteem [4]. WSLs usually occur on the smooth enamel surfaces [5] and are accompanied by loss of the mineral content underneath a pseudo-intact surface [6]. WSLs are characterized by a chalky white appearance as a result of light scattering [7].

One of the alternative treatment techniques of WSLs is using resin infiltration concept to cease the advancement of these lesions. This is achieved by the ability of a low viscosity resin to penetrate the enamel porosities thus stopping the progression of cariogenic acids and mineral loss [8]. Resin infiltration can mask the WSLs [6,8], owing to its refractive index which closely matches that of enamel; so it enhances the lesion translucency, moreover, it strengthens the compromised enamel prism structure [9]. However, this may lead to darker appearance of the infiltrated teeth, as patients became familiar with the whitish color of WSLs [10,11]. To overcome this, treatment with bleaching can be done prior to the resin infiltration [10] not after it, as resin can hamper the action of bleaching agents [11] as proved in an in-vitro study by Santos et al. [11].

Bleaching is considered one of the treatment modalities to disguise the WSLs as it decreases the dissimilarity between these lesions and enamel [12]. Despite this, it is not clear if the bleaching agent influences WSLs negatively or not [13]. Carbamide peroxide and hydrogen peroxide are the most commonly used bleaching compounds [14].

The long-term serviceability of resin infiltrated surface depends on its color stability [15], which is influenced by the material's polishability and its surface roughness [16]. Rough surfaces promote plaque accumulation leading to increased risk of demineralization [17].

The mineral content of the enamel is evaluated indirectly by the microhardness testing. It was found that bleaching negatively affects demineralized enamel microhardness [18] while resin infiltration has a positive impact [19].

Accordingly, the objective of this study was to evaluate the surface roughness and microhardness of human enamel with induced white spot lesions before and after application of resin infiltration material (ICON), bleaching agent and bleaching agent followed by resin infiltration material (ICON) and after acidic challenge.

MATERIAL AND METHODS

Specimens' preparation and grouping

A total of 30 sound human premolars extracted for orthodontic reasons within age group of 18-26 years were used in this study with the following exclusion criteria: demineralization lesions, stains, fluorosis, decay, enamel defects, or restorations. Teeth were collected from the department of Oral and Maxillo-Facial Surgery, Faculty of Dentistry, Ain Shams University, through the legal authorization from the patients and the study was approved by the Ethical Committee of the Faculty of Dentistry, Ain Shams University (FDASU - Rec M031807). Teeth were cleaned from blood under running water. All soft tissues and hard deposits were removed using a universal hand scaler (Dentsply, Maillefer, USA). Polishing of the teeth was then done using a rubber prophylaxis cup mounted on a low-speed handpiece with a mix of water and non-fluoridated oil-free pumice before preparation procedures [20]. Teeth were evaluated by a stereomicroscope (Olympus Stereozoom SZ 40 Microscope, Tokyo, Japan), where teeth with any of the exclusion criteria were not used in this study [10]. Teeth were then stored for a maximum of 1 month before use in 0.1% thymol solution at room temperature to prevent dehydration [17,19] and ensure aseptic conditions [17].

After removal of the teeth (n=30) from the thymol solution, each tooth was longitudinally sectioned bucco-lingually using a low-speed precision saw (Isomet 4000, Buehler, Lake Bluff, IL, USA) at 2500 rpm and 6 mm/min feed rate under copious water coolant, providing two halves from each tooth with a total number of 60 halves and assigned randomly into three experimental groups according to the treatment modality applied (n=20). Group I involved treatment with resin infiltration (ICON, DMG, Hamburg, Germany), group II involved treatment by bleaching (Opalescence Boost PF 40% HP, Ultradent Products, Inc., South Jordan, Utah, USA) and in group III specimens were subjected to bleaching followed by resin infiltration. For each treatment modality, 10 specimens were tested for surface roughness and another 10 for microhardness.

Each specimen was covered with 2 layers of transparent acid-resistant nail polish (Maybelline, New York, USA) for isolation of all surfaces of the specimen including the sectioned surface, except for a 4x4 mm^2 window [21] on the proximal (the mesial or distal) surface of each specimen, that was kept unprotected.

Induction of WSLs

All specimens used in this study (n=60) were put in a demineralizing solution containing acetic acid with pH 4.4 for 96 hours for the production of artificial WSLs in the window area with approximately 100–150 μm depth [22].

The demineralizing solution was prepared by adding 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, 0.05M acetic acid and adjusted at pH 4.4 with 1 M KOH [21,22]. Calculation of the total amount of the used demineralizing solution was done and 2 mL per mm^2 of the enamel was used [23]. Therefore, each specimen was immersed in a volume according to the equation: (4x4= 16 mm^2) x (2 mL) = 32 mL for 96 hours in a separate glass container and kept in an incubator at 37 °C with the renewal of the demineralizing solution every 24 hours during the whole demineralization period [24]. Each specimen was visually checked to make sure that the WSL was successfully created on the enamel surface [19].

Specimens were stored in artificial saliva after WSLs induction and was renewed daily [18]. Artificial saliva included hydrogen carbonate (22.1 mmol/L), potassium (16.1 mmol/L), sodium (14.5 mmol/L), hydrogen phosphate (2.6 mmol/L), boric acid (0.8 mmol/L), calcium (0.7 mmol/L), thiocyanate (0.2 mmol/L) and magnesium (0.2 mmol/L) in accordance with the formula of Göhring et al. [25]. The pH ranged from 7.4 to 7.8 [21,25].

Group I (Resin infiltration IC)

According to the manufacturer's instructions, the resin infiltration material (ICON) (DMG, Hamburg, Germany) (Table I) was applied to the window area of the specimens [26]. The window areas with the induced WSLs were cleaned with a polishing brush and water, etched for 2 minutes using 15% hydrochloric acid (Icon Etch, DMG, Hamburg, Germany) followed by rinsing with air-water spray for 30 seconds. The induced WSLs were dried for 10 seconds by air blowing. Ethanol (Icon Dry, DMG, Hamburg, Germany) was then applied for 30 seconds then air-blown for 10 seconds. After this, resin infiltrant (Icon Infiltrant, DMG, Hamburg, Germany) was applied using a sponge applicator supplied by the Icon kit and left for 3 minutes. Removal of excess resin was done by using a cotton roll before being light-cured for 40 seconds by a LED light-curing unit of 1200 mW/cm^2 output (Elipar S10, 3M ESPE, Germany) with a 10 mm diameter curing tip from the window area. The light curing intensity was regularly checked using a radiometer (Model 100 curing radiometer, Kerr, USA). The resin infiltrant was applied again for 60 seconds and light-cured for 40 seconds. The infiltrated surfaces were polished each for 20 seconds using fine and superfine flexible discs (Sof-Lex, 3M ESPE, St Paul, MN, USA) with low speed [10].

Group II (Bleaching OPF)

An in-office bleaching agent (Opalescence Boost PF 40% HP, Ultradent Products, Inc., South

Table I - Materials used in the study, their manufacturers, composition and lot numbers

Materials	Manufacturers	Composition	Lot number
ICON	DMG,Hamburg, Germany	**Resin infiltration kit consisting of:** -Icon-Etch:15% hydrochloric acid; -Icon dry: 99% ethanol; -Icon-infiltrant:TEGDMA-based resin, initiators, and additives	788939
Opalescence Boost PF 40% HP	Ultradent Products, Inc., South Jordan, Utah, USA	In-office bleaching agent consisting of 2-barrel jet mix; One barrel contains 1.1% NaF and 3% KNO3, along with a unique chemical activator, which is combined with a second barrel containing hydrogen peroxide.	BGB23

Jordan, Utah, USA) (Table I) was applied on the window area. The bleaching system included two syringes: one syringe contained the chemical activator, while the other contained hydrogen peroxide (HP). The two components were mixed according to the manufacturer's instructions before being applied. A polishing brush and water were used to clean all the specimens, then a uniform continuous layer of approximately 0.5-1.0 mm of the activated gel was applied for 20 minutes as recommended by the manufacturer then rinsed with air-water spray [10].

Group III (Bleaching followed by resin infiltration OPF/IC)

In-office bleaching agent followed by resin infiltration material was applied on the window area of the specimens as previously described. ICON was applied 14 days after the application of the OPF [10].

Acidic challenge

Acidic challenge was performed after treatment to simulate the effect of acidic oral environment on the surface roughness and microhardness of the treated surfaces. All the specimens of the three experimental groups (n=60) were immersed for 96 hours in separate glass containers in the same demineralizing solution which was used for the induction of artificial WSLs previously described and was changed daily [21,22,24].

Measurement of surface roughness

Surface roughness was measured by the tapping mode of the atomic force microscope (AFM) (5600LS, Agilent technologies, Santa Clara, CA, USA). Images were obtained with a slow scan rate of 0.4 lines/second and a resolution of 256x256 pixels per image was chosen. Images with scan size 20x20 μm were taken for each specimen and the whole image was used for surface characterization. Quantitative information concerning the surface roughness (Ra) was obtained from the AFM images of the specimens using Gwyddion 2.54 software (An open-source software for SPM data analysis, Brno, Czech Republic) [27]. The average of four readings of Ra for each image was calculated.

Specimens were tested at baseline (before demineralization), after demineralization, after different treatment protocols, and after acidic challenge.

Measurement of microhardness

Before any microhardness measurement, a cylindrical plastic mold of 25 mm in diameter and 10 mm in height was painted by a separating medium and placed on a glass slab to obtain a flat surface. Cold cure acrylic resin was mixed in a glass container according to the manufacturer's instructions then packed in the mold and the specimen was mounted on top of the acrylic block with the window area facing upward. Excess acrylic material was removed, and another glass slab was placed on the top of the sample to make sure that the tooth is parallel to the horizontal plane.

After setting of cold-cure acrylic resin, the whole block was painted with 2 coats of colored acid-resistant nail polish (Maybelline, New York, USA) except for the window area to prevent any reaction of the acrylic material with the demineralizing solution. After the dryness of the nail polish, the block was numbered by a waterproof pen.

Microhardness was measured using digital Vickers hardness tester (Wilson®, Tukon™ 1102, Buehler, Lake Bluff, IL, USA). A static load of 100gf was applied to the surface of the window area for 10 seconds as recommended by Prajapati et al. [28]

To ensure the accuracy of the measurements, indentations were done on flat test points of the window which were determined using the microscope attached along with the microhardness tester at 10X and 50X magnifications. For each measurement, three indentations were done with a distance of 100-μ from each other [28].

Digital readings were noted for each indentation and the mean surface hardness value of three indentations was calculated for each specimen and reported as Vickers hardness number (VHN).

Specimens were tested at baseline (before demineralization), after demineralization, after different treatment protocols, and after acidic challenge.

Statistical analysis

Numerical data were tested for normality using Shapiro-Wilk test and showed normal distribution. Surface roughness and microhardness results were shown as mean and standard deviation (SD) values and analyzed using one-way ANOVA followed by Tukey's post hoc test for intergroup comparisons and one-way repeated measures ANOVA followed by Bonferroni's post hoc test for intragroup comparisons.

The level of significance in all tests was set at $p \leq 0.05$. Statistical analysis was carried out using the R statistical analysis software version 4.0.3 for Windows (Bunny-Wunnies Freak Out, 2020, Austria) [29].

RESULTS

The results of this study are shown in Tables II and III and Figure 1.

Surface roughness

The results of surface roughness are presented in Table II. One-way ANOVA revealed insignificant difference in Ra values between the three tested groups ($p>0.05$) at baseline and after the induction of WSLs. However, there was a significant difference ($p<0.001$) between the three groups after treatment and after acidic

Table II - Mean and Standard deviation (SD) values of Ra measured in nanometers for the three tested groups (Resin infiltration (IC), Bleaching (OPF) and Bleaching followed by resin infiltration (OPF/IC)) before demineralization, after demineralization, after treatment and after acidic challenge

Measurement time	Ra (Mean ± SD)			p-value
	IC	OPF	OPF/IC	
Before demineralization	12.14 ± 0.51^{Ac}	13.86 ± 0.48^{Ad}	13.82 ± 0.38^{Ad}	**0.073ns**
After Demineralization	23.62 ± 0.38^{Aa}	24.82 ± 0.38^{Ac}	21.80 ± 0.39^{Ab}	**0.098ns**
After treatment	14.94 ± 0.32^{Bb}	30.88 ± 0.41^{Ab}	15.22 ± 0.47^{Bc}	**<0.001***
After acidic challenge	24.30 ± 0.72^{Ba}	40.30 ± 0.64^{Aa}	24.36 ± 0.85^{Ba}	**<0.001***
p-value	**<0.001***	**<0.001***	**<0.001***	

Different Uppercase letter indicates significance difference within row. Different Lowercase letter indicates significant difference within column ($p<0.05$). *Significant difference.

Table III - Mean and Standard deviation (SD) values of Vickers hardness number (VHN) for the three tested groups (IC, OPF and OPF/IC) before demineralization, after demineralization, after treatment and after acidic challenge

Measurement time	VHN (Mean ± SD)			p-value
	IC	OPF	OPF/IC	
Before demineralization	354.40 ± 4.07^{Aa}	351.94 ± 4.73^{Aa}	357.74 ± 16.88^{Aa}	**0.684ns**
After Demineralization	156.64 ± 20.16^{Ab}	144.80 ± 9.95^{Ab}	164.66 ± 13.82^{Ab}	**0.159ns**
After treatment	288.86 ± 27.04^{Aa}	152.52 ± 19.38^{Bb}	315.72 ± 6.11^{Aa}	**<0.001***
After acidic challenge	191.04 ± 27.52^{Ab}	103.52 ± 13.33^{Bc}	203.30 ± 13.46^{Ab}	**<0.001***
p-value	**<0.001***	**<0.001***	**<0.001***	

Different Uppercase letter indicates significance difference within row. Different Lowercase letter indicates significant difference within column ($p<0.05$). *Significant difference.

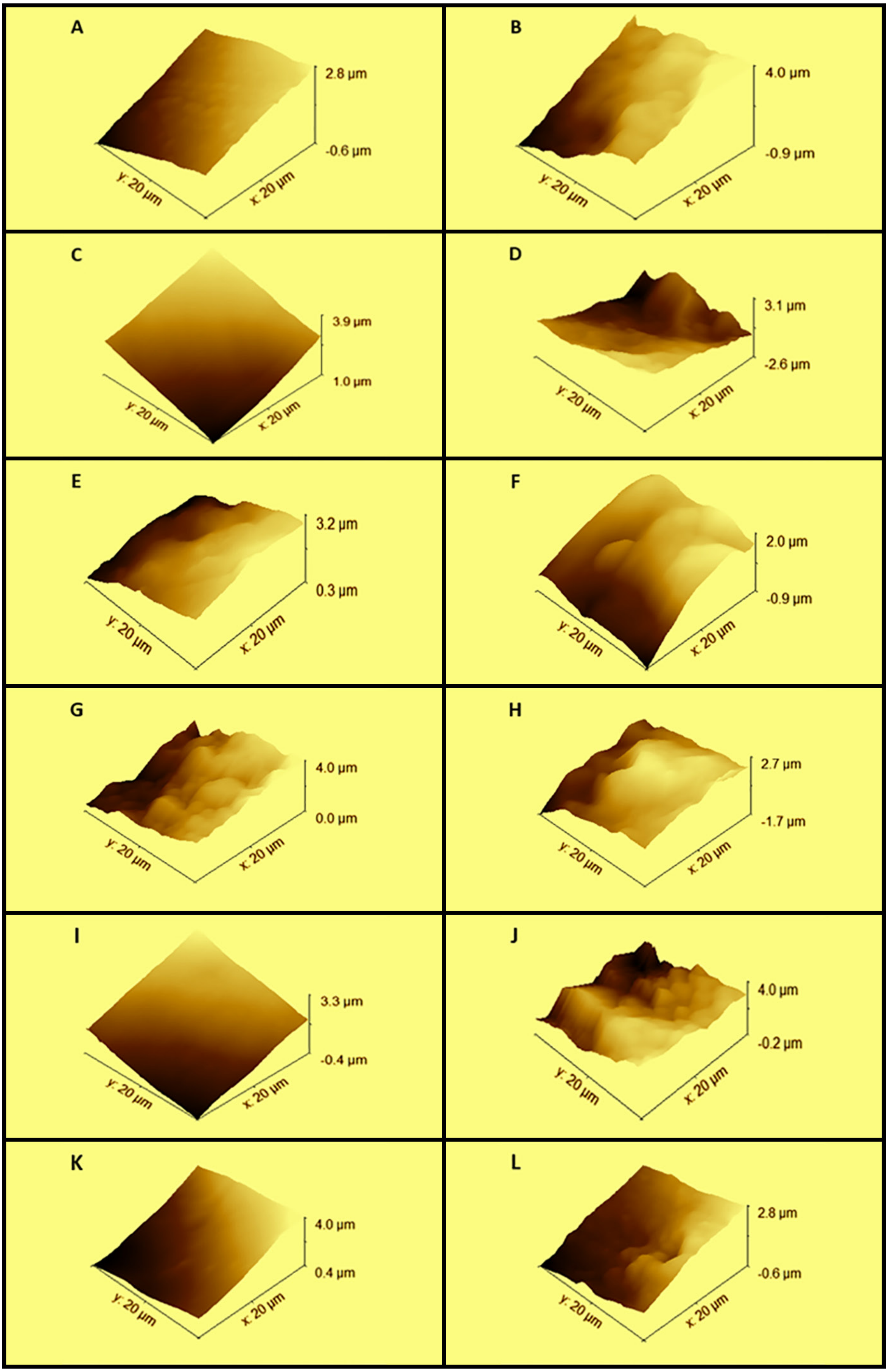

Figure 1 - Atomic force microscopy tapping mode 3D images (20x20 µm) showing the surface of enamel of representative samples from groups I (A-D), II (E-H) and III (I-L). (A, E, I) Represent before demineralization; (B, F, J) Represent after demineralization; (C) Represents after resin infiltration; (G) Represents after bleaching; (K) Represents after bleaching followed by resin infiltration; (D, H, L) Represent after acidic challenge.

challenge where post hoc pairwise comparisons showed Ra value of OPF group to be significantly higher than those of the other two groups.

Repeated measures ANOVA showed a statistically significant difference in Ra values within the same group ($p<0.05$), where the highest value was shown after acidic challenge followed by that after the induction of WSLs, after treatment and the lowest Ra values were recorded at baseline for groups I and III. For group II, the highest value was shown after acidic challenge followed by after treatment then after the induction of WSLs and the lowest Ra value was recorded at baseline.

Atomic force microscopy tapping mode 3D images showing the surface of enamel of representative samples from groups I, II, and III are presented in Figure 1A-L. Top view of normal pattern of enamel prisms before demineralization is shown in Figure 1A, E, I. Increased surface roughness due to interprismatic dissolution after demineralization is represented in Figure 1B, F, J. Smooth surface of the resin covering the enamel after resin infiltration in groups I and III is shown in Figure 1C, K. Rough surface due to the effect of the bleaching agent is shown in Figure 1G. Increased surface roughness after acidic challenge is presented in Figure 1D, H, L and disruption of resin layer is shown in Figure 1D, L.

Microhardness

The results of microhardness are presented in Table III. One-way ANOVA revealed insignificant difference in VHN values between the three tested groups ($p>0.05$) at baseline and after the induction of WSLs, while there was a significant difference between them ($p<0.001$) after treatment and after acidic challenge where post hoc pairwise comparisons showed VHN values of IC and OPF/IC groups to be significantly higher than that of OPF group.

Repeated measures ANOVA showed significant difference in VHN values within the same group ($p<0.05$), where the highest value was shown at baseline followed by after treatment then after acidic challenge and the lowest values were recorded after the induction of WSLs for groups I and III. For group II, mean VHN value measured before demineralization was significantly higher than those measured at other intervals. Moreover, there was no significant difference between mean VHN values measured after demineralization and after treatment where both were significantly higher than that measured after acidic challenge.

DISCUSSION

The primary goal for treatment of WSLs is to stop their progression and enhance esthetics by decreasing the opacity of these lesions. This can be achieved by the resin infiltration (ICON) which seals the microporosities that act as diffusion channels for dissolved minerals and acids [30]. Resin infiltration, being a micro-invasive treatment modality [30], has gained a good reputation as an effective option for treating the WSLs, particularly in orthodontic patients [31].

Acid etching is required before resin infiltration because of the presence of the pseudo-intact surface of WSL which hinders the penetration of resin owing to its insufficient porosity. Etching with conventional phosphoric acid gel does not produce porosities sufficient for resin infiltration, while 15% HCl (Icon etch) is successful in partially removing this pseudo-intact surface resulting in broader surface porosities allowing resin infiltrating the lesion's more porous subsurface body, resulting in increased resin penetration depth [32]. Meyer-Lueckel et al. [33], found that 15% HCl for 120 seconds removes about 40 μm of the pseudo-intact surface of WSL [33]. It was found that etching once was sufficient to open up the pores of the WSLs without tooth weakening [34].

Ethanol 99% (Icon dry) was used in our study for the removal of water found inside the microporosities of the body of the WSL for proper penetration of resin [28]. By decreasing the viscosity and contact angle, ethanol increases the resin penetration coefficient [24]. According to the manufacturer's instructions, the infiltrant resin had been applied twice. The second application of resin is recommended for occlusion of the space caused by the resin polymerization shrinkage resulting from the first time application [28].

Paris et al. [35], suggested that excessive resin is a disadvantage clinically as it leads to the formation of biofilm retention sites and subsequent new formation of caries lesions [35], therefore it was essential to remove the excess resin prior to light-curing [1]. Clinically, final polishing after polymerization of the infiltrant material is very important to avoid discoloration of superficial unpolymerized resin components [36].

One of the most trendy esthetic treatments is bleaching, which gives the patients a more perfect appearance and smile [18]. It has been considered a treatment modality to camouflage WSLs that are seen in patients after orthodontic treatment [1]. This was attributed to the ability of bleaching to diminish the contrast between WSL and the adjacent sound enamel [10]; thus improving the esthetic appearance of these patients [18]. In this study, in-office bleaching (40% hydrogen peroxide gel) was used as it is more practical and predictable compared to home bleaching [37].

The active bleaching agent in the bleaching products is hydrogen peroxide which generates free radicals after its decomposition. The free radicals are effective oxidizers and diffuse through both enamel and dentin and produce small molecules by the cleavage of the double bonds of the pigmented compounds, thus resulting in the whitening effect [12,18]. It was verified that the rise in the concentration of peroxide contributes to a reduction in the pH of the solution of bleaching. This acidic medium leads to dental hard tissues demineralization as the amount of free radicals that can invade the enamel organic part is increased [38]. Another protocol has been suggested for the treatment of WSLs that involves bleaching before applying resin infiltration to enhance the esthetic outcome and the patient's satisfaction [10,11].

The same demineralization protocol was used for induction of WSLs and for the acidic challenge to compare the resistance of sound enamel before induction of WSLs to that of the treated WSLs.

In this in vitro study, surface roughness and microhardness were selected for evaluation because changes in them have a direct effect on the durability of treatment [39] and may cause problems such as color change of the treated surfaces and accumulation of plaque with subsequent caries formation [31]. The roughness of the treated surfaces depends on the type of materials used [17].

In this study, the AFM was used as it is a non-invasive method that involves minimal sample preparation, offers 2D and 3D images, enables the sample to be reevaluated [40], offers higher resolution images, and does not have SEM drawbacks such as coating and vacuum [41]. The tapping mode of the AFM was used in this study to avoid surface dragging or any unwanted tip-sample interactions as that produced by contact mode [42].

In this study, there was no statistically significant differences in Ra values between the three groups after induction of WSLs. This showed that the demineralization solution produced uniform artificial white spot lesions in the study which was in agreement with Yazkan et al. [43]. Ra values after demineralization were significantly increased compared to before demineralization in the three groups, this increase might be attributed to an increase in enamel porosity resulting from interprismatic dissolution as shown in Figure 1B, F, J and accurate detection of Ra changes by the AFM [43].

In agreement with the findings of Enan et al. [31] and Yazkan et al. [43] after ICON infiltration, there was significant reduction in Ra values compared to after demineralization [31,43] as shown in Figure 1C. However, the roughness of resin infiltrated lesions was still significantly higher than that of sound enamel, this was in agreement with the findings of Qasim et al. [24] and Yazkan et al. [43]. This might be due to excess resin was not completely removed after polishing [17,35,43] or might be due to microporosities not completely filled by resin infiltrant due to polymerization shrinkage [39].

Ra values in the IC group increased significantly after the acidic challenge as shown in Figure 1D, this finding was in agreement with Yazkan et al. [43]. This might be attributed to the fact that TEGDMA is very hydrophilic and that leads to its degradation in the oral environment, therefore it is considered as a non-resistant material [44]. There was insignificant difference in Ra values between groups IC and OPF/IC as in both groups the surface was covered by the same resin as shown in Figure 1C,K and polished in the same manner.

Ra increased significantly after bleaching compared to after induction of WSLs as shown in Figure 1G and this was in agreement with the findings of Rodrigues et al. [38], this might be due to the low pH of the bleaching solution creating an acidic medium which leads to demineralization of dental hard tissues as the amount of free radicals that can invade the enamel organic part is increased [38]. Moreover, Ra values further increased after the acidic challenge as shown in Figure 1H which is probably due to further demineralization.

WSLs have already been shown to have lower microhardness than the surface of sound enamel [28], which was in agreement with the findings of this study. The reduction in microhardness after induction of WSLs was attributed to the softening of enamel by chemical dissolution of enamel rods and the formation of porosities [30].

The microhardness of WSLs increased significantly with the infiltration of resin, in accordance with Torres et al. [45] and Enan et al. [31]. The ability of ICON to increase the microhardness can be explained by the low viscosity of the resin, which allows its deep penetration into the WSLs immediately after treatment [31,39] and the resin encapsulation of the remaining hydroxyapatite (HA) crystals, giving a uniform resin-HA complex of high surface hardness value [31]. Also, the repeated resin application improved the microhardness as it compensated for the polymerization shrinkage and filled the porosities inside the body of the lesion [41,43].

The reduction in surface microhardness after the acidic challenge might be due to the dissolution of the remaining minerals in the body of the lesion that was not completely infiltrated with the resin [45] or might be due to the hydrolytic degradation of TEGDMA [44]. This reduction in hardness value after the acidic challenge was in agreement with the findings of Yazkan et al. [43].

The microhardness test is a simple and commonly used method for evaluating the mechanical properties of enamel after bleaching, and the measurements indicate the mineral content of the bleached samples [18].

The effect of bleaching as a treatment on the surface hardness of enamel is controversial. Some studies showed a reduction in hardness, while other studies revealed no significant change. This discrepancy might be due to the duration of exposure to the bleaching agent, the pH of the bleaching solution or the treatment procedure itself [38].

Despite the low pH of the bleaching agent which contains 40% hydrogen peroxide, the hardness of the WSLs treated with the bleaching agent (OPF group) was not reduced and there was insignificant difference between mean VHN values. This could be related to the fluoride content of the bleaching agent used. This was in agreement with the findings of Horuztepe et al. [10]. Cavalli et al. [46] reported that the mineral loss was decreased by the addition of fluoride and calcium to the bleaching agents [46]. Furthermore, it has been reported that a fluoridated bleaching gel minimizes the time necessary for the recovery of bleached enamel hardness by the formation of fluoridated hydroxyapatite which is beneficial for tooth surface remineralization compared to unfluoridated bleaching gel [47]. Moreover, enamel demineralization after acidic challenge resulted in a significant reduction in microhardness.

For the OPF/IC group, the microhardness was improved in a way that there was no statistically significant difference in the microhardness values compared to before demineralization. Moreover, there was statistically significant increase compared to after demineralization due to the combined positive effect of the fluoridated bleaching agent and the resin infiltrant, this finding was in agreement with the findings of Horuztepe et al. [10].

Artificial saliva might also have contributed to the increase of the enamel microhardness by supplying calcium and phosphate ions. The pH of artificial saliva in which the samples were immersed ranged from 7.4 to 7.8, which was well above the critical pH (5.5) of demineralization of enamel [48].

Nevertheless, long-term clinical and laboratory studies are required to compare the three treatment modalities regarding their efficiency in decreasing surface roughness and increasing microhardness of the WSLs and their resistance to acidic challenges.

CONCLUSIONS

Within the limitations of this study, the following could be concluded:

- Resin infiltration and bleaching followed by resin infiltration reduced the surface roughness and enhanced the microhardness of the WSLs;
- The three treatment modalities failed to resist the acidic challenge resulting

in increasing the surface roughness and reducing the microhardness.

Author Contributions

RHET: conceptualization, methodology, investigation, writing. MSN: formal analysis and supervision. DIEK: writing-reviewing and editing.

Conflict of Interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Regulatory Statement

This study was conducted in accordance with all the provisions of the Ethical Committee of the Faculty of Dentistry, Ain Shams University. The approval code for this study is: FDASU - Rec M031807.

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Date submitted: 2021 May 10
Accept submission: 2021 July 13