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Comparative Efficacy of Micro-Invasive Resin Infiltration Techniques on Acid Resistance and Enamel Microhardness in Orthodontic-Induced White Spot Lesions: An In Vitro Study.

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ABSTRACT

Background: White spot lesions (WSLs) affect 38–96% of orthodontic patients. They form around brackets within 4 weeks due to plaque accumulation in difficult to clean areas, leading to aesthetic issues and compromised treatment outcomes.

Objectives: This research was conducted to compare the effect of Icon versus composite resin sealant as resin infiltration systems on the ability to protect orthodontic induced WSLs against acid attacks.

Materials and methods: The study involved 120 premolars and 40 premolars from them were divided into 2 control groups (n=20): Group (1) un-demineralized enamel (positive control), Group (2) untreated WSLs (negative control). The remaining 80 premolars were divided into two treatment groups according to the material used in WSLs treatment (n=40); Group (3) Icon, and Group (4) Permaseal composite resin sealant. Groups (3 and 4) were further subdivided into two subgroups (n=20): tested before and after the acid exposure (Coca-Cola). Vickers microhardness evaluation was performed before demineralization, after demineralization, after Icon and Permaseal treatment, and after acidic exposure.

Results: The Icon and Permaseal groups exhibited significantly higher microhardness values than the negative control group but significantly lower than the positive control group. Nevertheless, a non-significant difference was found between them. However, the acidic exposure significantly decreased the microhardness values in the Icon and Permaseal subgroups, while the Permaseal subgroup showed the significantly lowest values. The Icon subgroup showed higher microhardness values compared to the negative control group, while the Permaseal subgroup showed non-significant higher difference.

Conclusions: Both Icon resin infiltration and Permaseal composite sealant effectively improved the microhardness of artificially induced orthodontic WSLs compared to untreated lesions. However, Icon resin infiltration exhibited superior resistance to acidic exposure, maintaining significantly higher microhardness post-acidic challenge than Permaseal.

Keywords: Orthodontic white spot lesions, Icon resin infiltration, composite sealant, acid resistance, microhardness

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1. INTRODUCTION

Dental caries is a prevalent global disease with a dynamic nature (1). A cyclical imbalance between demineralization and remineralization—triggered by acid production from cariogenic bacteria—causes subsurface enamel porosities that manifest as white spot lesions (WSLs). These early carious lesions remain reversible if the demineralization-remineralization balance is restored through appropriate intervention, allowing remineralization of the enamel and resolution of the lesion. (2).

Indeed, the equilibrium within the oral cavity is altered by fixed orthodontic appliances. Patients wearing orthodontic braces have demonstrated 2-3 times more plaque buildup than participants not wearing braces (3). According to previous research, teenagers who use fixed orthodontic appliances have a greater chance of developing enamel demineralization because these appliances upset the streptococcus mutans equilibrium in their mouths (4).

Furthermore, WSLs are a major iatrogenic complication of fixed orthodontics, affecting 38–96% of patients. They develop within 4 weeks of bracket placement, increasing from 11% immediately post-bonding to 38% at 6 months and 46% at 12 months (5). The development of white spot lesions (WSLs) and dental caries is a multifactorial process influenced by host susceptibility—including genetic predisposition and enamel quality—the amount and activity of cariogenic bacteria, salivary composition and flow, oral hygiene, and dietary habits. Factors like poor oral hygiene, low salivary flow, and high carbohydrate intake all contribute to an environment that promotes lesion formation and caries progression.(6).

Additionally, fixed orthodontic appliances alter the oral microbiome by creating niches that promote cariogenic bacteria. Complex brackets increase plaque retention at bracket-adhesive-enamel junctions, hard to clean by regular hygiene (7,8). After placement, Streptococcus mutans initially decreases, then rises above baseline within three months, while lactobacilli also increase in high-caries-risk patients, indicating elevated caries risk (9–11). The pH changes occur with compromised oral hygiene and increased plaque retention around orthodontic attachments. Treatment duration is a critical risk factor, as demineralization progresses throughout orthodontic therapy and becomes more pronounced in the second year (12).

Currently, remineralization is a non-invasive strategy for early carious lesions, marking a significant advancement in clinical management (13). Remineralization is effective yet esthetically limited due to superficial action; its success is time-sensitive and contingent on early intervention (14).

On the other hand, resin infiltration is a minimally invasive technique that is used to manage WSLs. It is a promising approach that stabilizes demineralized lesions and improves the microhardness of dental tissues. Moreover, the resin effectively masked WSLs owing to its refractive index closely matching that of enamel.(15).

Resin infiltration aims to alter light scattering by filling lesion porosities with a resin of refractive index approximating healthy enamel, thereby masking the defect [16]. Infiltration Concept (Icon) is a resin product developed in Germany to treat incipient lesions. with RI (1.46) close to that of healthy enamel (1.62), Thus, the enamel–porosity contrast becomes negligible, rendering the lesion visually indistinguishable from adjacent enamel. [17].

Furthermore, infiltrating non-cavitated enamel lesions with various resins markedly diminishes white spot visibility and enhances resistance to demineralization [18]. Given the routine availability of adhesive systems and fluid resinous materials (e.g., composite sealants) in dental practice, evaluating their potential as alternatives to Icon infiltrant is clinically relevant. These materials could serve multiple purposes, including color masking, penetrating the pores of WSLs to prevent further caries progression, and arresting the progression of caries [19].

Long-term stability of restorative materials depends on their resistance to chemical and physical cyclic degradation within the oral environment [20]. Therefore, restorative material evaluation should simulate oral conditions, particularly pH fluctuations [21]. Moreover, dental biofilm pH affects both enamel integrity and the chemical–physical properties of restorative materials [22].

The oral environment is highly dynamic, influenced by fluctuating salivary flow, diverse microbial populations, and continuous pH changes[23]. Although resin infiltration arrests WSLs, persistent physicochemical fluctuations continue to challenge both enamel and restorative materials, potentially inducing structural alterations in both substrates [23]. To simulate these challenges and evaluate the acid resistance of resin infiltrants, pH cycling models are widely employed in research [24].

However, the question remains whether resin-treated enamel can offer long-term protection against common oral cavity changes, such as pH changes. Additionally, the impact of pH fluctuations on resin-infiltrated enamel surface properties remains not well-established [18,19].

2. OBJECTIVES

This research was conducted to compare the effect of Icon versus composite resin sealant as resin infiltration systems on the ability to protect orthodontic induced WSLs against acid attacks. The null hypothesis was that the resin infiltrations would increase the microhardness of the WSLs, and the acidic exposure wouldn't impact the microhardness of infiltrated WSLs.

3. MATERIALS AND METHODS

Materials:

The composition and manufacturers of the materials employed in this study are presented in (Table 1).

Sample size calculation:

As per de Cerqueira et al. [24], the sample size was estimated using G*Power v3.1 for Windows. The sample size was determined based on Mixed Model ANOVA considering the baseline and repeated measures to assess the timing factor (repeated measures) and grouping factor (4 groups). It was calculated based on the following assumed inputs; Effect Size ($f = 0.19$), Number of Groups (4), Number of Measurements (2) at a Power ($1-\beta = 0.95$), Significance Level ($\alpha = 0.05$) = 0.05, Correlation Among Repeated Measures (0.5), Non-sphericity Correction ($\varepsilon = 1$), and was found that sample size would be 20 into each group (n=20).

TABLE 1. Brand name, description, composition, Lot number, and company of the used materials

Product brand name	Product description	Composition		Lot number	Company
Icon	Smooth surface resin infiltration	Icon etch	15% hydrochloric acid (HCL), pyrogenic silicic acid, and surface-active substances	203758	DMG, Hamburg, Germany
		Icon dry	99% ethanol		

		Icon infiltrant	TEG-DMA Methacrylate-based resin matrix, initiators and additives		
Permaseal	Composite resin sealant	BIS-GMA resin 60%, TEG-DMA, 40%, 2-dimethylaminoethyl Methacrylate <3%		BHPG7	Ultradent Products, Inc., South Jordan, UT, USA

TEGDMA: Triethylene glycol dimethacrylate, Bis-GMA: Bisphenol glycidyl methacrylate.

Teeth selection and ethical regulations:

A total of 120 premolars, extracted for orthodontic or periodontal indications, were selected for the study. Teeth were free from cracks, caries, or restorations. The teeth were obtained from the outpatient surgery clinics of the Faculty of Dental Medicine, Al-Azhar University, Egypt, Cairo, and the ethics committee's approval (No. 1003/466). The calculus, tissue deposits, and stains were removed thoroughly with a sharp scaler, rubber cup & non-fluoridated polishing paste. After that, the teeth were stored in normal saline at room temperature from the day of extraction until used, and the water was changed daily.

Grouping:

A total of 120 premolars were used in this study and 40 premolars from them were randomly divided into 2 control groups (n=20) as the following: Group (1) un-demineralized sound enamel surface (positive control), Group (2) untreated WSLs (negative control).

While the remaining 80 premolars were divided randomly into 2 treatment groups according to the material used in WSLs treatment (n=40); Group (3) Icon treatment of WSLs, and Group (4) Permaseal composite resin sealant treatment of WSLs.

The treated WSLs groups (3 and 4) were further subdivided into two subgroups (n=20): Subgroup (A) tested before the acid exposure and Subgroup (B) tested after the acid exposure (Coca-Cola).

Decalcification:

For the removal of the root part, a low-speed diamond disc including water cooling was used to segment each premolar 1 mm beyond the cemento-enamel junction. The selected premolars were coated with nail varnish, leaving a 4 × 4 mm window on the buccal surfaces. Sectioned root area were sealed with cyanoacrylate to avoid penetration of demineralization through the root canals [(27)]. The baseline microhardness assessment was performed on the exposed sound enamel windows for 20 premolars in Group 1: un-demineralized sound enamel (positive control group).

After that, the artificial WSLs were created by immersion of every premolar in 32 mL of a demineralizing liquid, including 50 mM acetate buffer solution and 1.28 mM $Ca(NO_3)_2$_$4H_2O$, 0.74 mM $(NaH_2PO_4).2H_2O$, and 0.03 ppm F at pH 5.0 for 24 hours at 37 8C. Afterward, teeth were removed from the demineralizing liquid and completely rinsed utilizing deionized water [(23)]. Each tooth was then embedded in self-curing acrylic resin using a Teflon mold on its lingual surface while the buccal surface faced outward to position the samples during microhardness assessments. Then, the second microhardness assessment was performed on 20 premolars in Group 2: the created WSLs (negative control group) on the exposed enamel windows.

Resin sealants (Icon and composite sealant) application:

The Icon manufacturer's instructions were followed for applying Icon and composite sealant (Permaseal) [(28)]. The first step was the Icon-Etch application, which lasted for 2 minutes. After that, the lesions were sprayed with water for 30s, then dried with compressed air for 10s. The second step was applying Icon-Dry to the etched areas and letting set for 30s, and then the lesions dried thoroughly with air for 10s. The last step was the application of the Icon-Infiltrant resin and composite sealant as resin sealants on the etched area, which were then rubbed continuously for 3 minutes. The excess resin was removed and then light-cured for the 40s using the LED light curing unit (Elipar S10, 3M ESPE, St Paul, Minnesota, US). All the resin sealants were applied again and rubbed for 60s. The excess resin was removed and light-cured for the 40s. Finally, after all materials were applied, the treated WSLs were polished with the rubber cup and polishing paste. Then, the third microhardness assessment was performed on 40 premolars (20 treated with Icon and 20 treated with Permaseal) from the total of 80 premolars with treated WSLs which represented the Subgroup (A) of the resin-infiltrated WSLs in both groups (Icon and Permaseal).

Acidic exposure:

The remaining 40 premolars with treated WSLs (20 Icon and 20 Permaseal) were immersed in the 100 ml Coca-Cola solution for 5 minutes, 5 times daily, for 30 days [26]. The waking period is assumed to be approximately 12 hours a day [29]. Samples were stored in artificial saliva between immersion cycles; beverages were applied at consumption temperature (± 4 °C) [26]. Then, the fourth microhardness assessment was performed on the Subgroup (B) of resin-infiltrated WSLs in both groups (Icon and Permaseal).

Microhardness assessment:

The microhardness test was performed at four different time intervals: one at baseline for the positive control (sound enamel) and one after artificial demineralization for the negative control (demineralized enamel). For samples treated with Icon and composite sealant, testing was conducted post-treatment and subsequent to acidic exposure. The research's illustrative diagram is shown in (Figure 1).

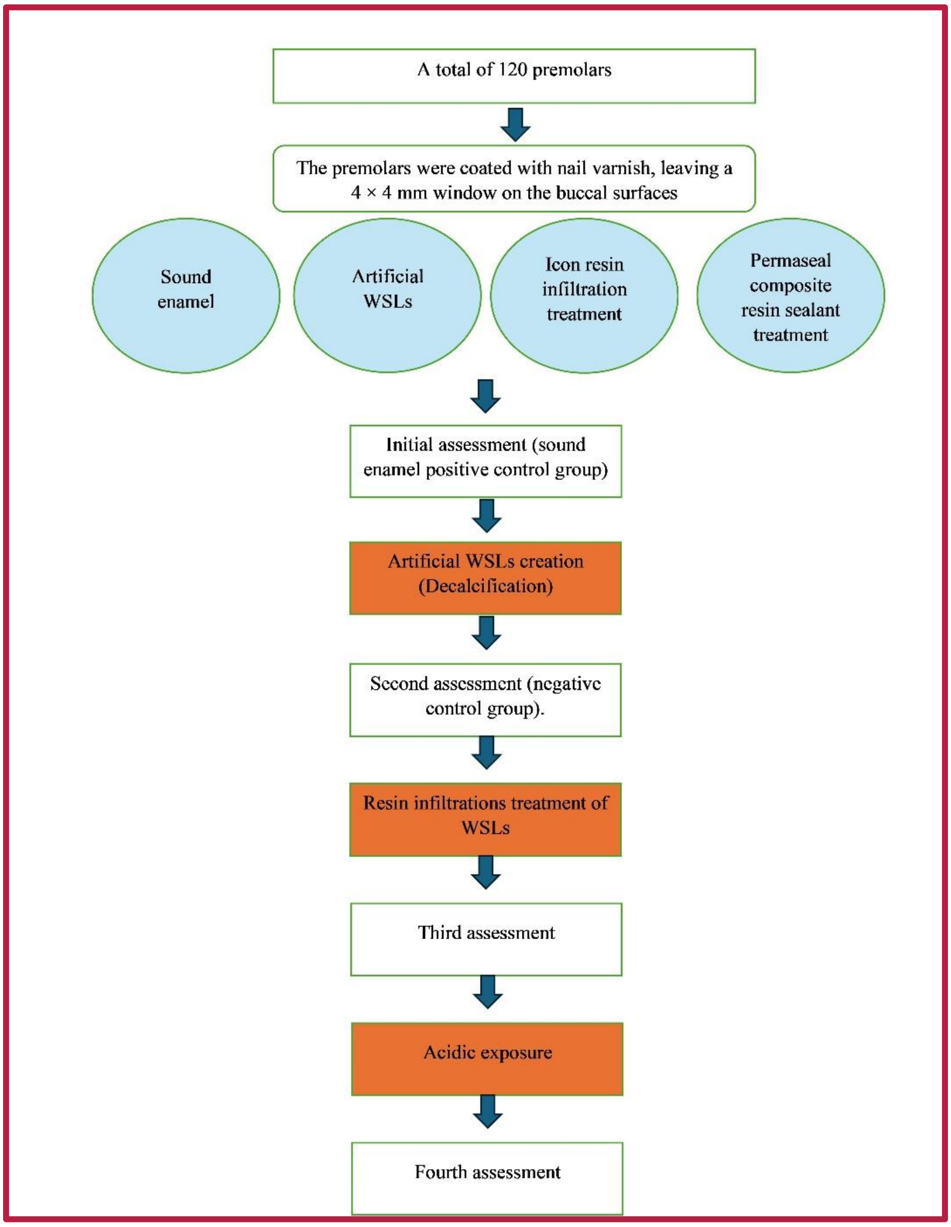

FIGURE 1. The research's illustrated diagram

Microhardness was measured using a Digital Display Vickers Microhardness Tester (Model HVS-50, Laizhou Huayin Testing Instrument Co., Ltd., China) fitted with a Vickers diamond indenter and a 20× objective lens. A 200 g load was applied for 10 seconds to each specimen surface, with three indentations made equidistantly by 0.5 mm in a circular pattern. The diagonal lengths of indentations were measured with a built-in scaled microscope .Microhardness value was obtained using the following equation: $HV = 1.854\ P/d^2$ where HV is Vickers hardness in Kgf/mm^2, P is the load in Kgf, and d is the average length of the two diagonals in mm [(23)].

Statistical analysis

A program for statistical analysis (SPSS 12.0, SPSS, Chicago, Illinois) was used to conduct the analysis. The threshold for significance was fixed at $p \leq 0.05$. The Shapiro-Wilk test for measuring the normality distribution was used to examine the information gathered for normality. Since the microhardness data had a normal distribution, the variation in mean microhardness across the studied groups was examined using an analysis of variance (ANOVA). Materials were compared using an independent t-test following an acidic exposure. While treatments prior to and following the acidic exposure were compared using the t-test.

4. RESULTS:

Microhardness of the different groups before the acidic exposure:

The results demonstrated that both Icon and Permaseal groups exhibited significantly elevated mean microhardness compared to the negative control (untreated WSLs), yet remained inferior to the positive control (sound, un-demineralized enamel). Nevertheless, a non-significant difference (p = 0.33) was found between the Icon and Permaseal groups, and higher mean microhardness values were achieved with the Icon group (Table2 and Figure 2).

TABLE 2. Microhardness values for the different groups before the acidic exposure (One-way ANOVA)

Group	Mean	SD	p-value
Positive control group (sound enamel surface)	181.3^{A}	17.35	<0.001*
Negative control group (untreated WSLs)	129.20^{C}	9.13	
Icon resin infiltration group	169.75^{B}	12.97	
Permaseal composite resin sealant group	165.80^{B}	12.53	

*; Significant at p<0.05. Different litters mean statistically significant.

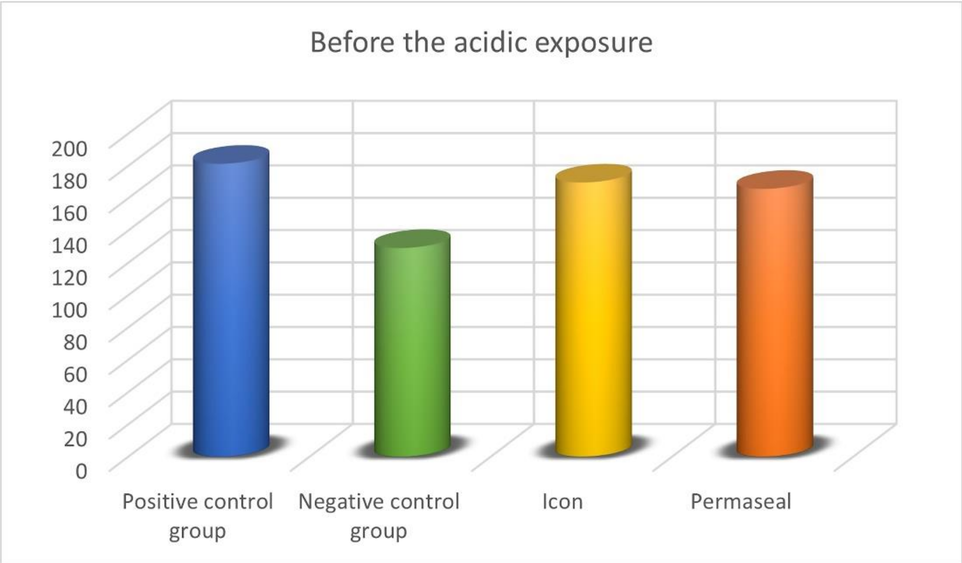

FIGURE 2. Bar chart showing the microhardness values for the different groups before the acidic exposure

Effect of acidic exposure on both treated groups:

After the acidic exposure, the mean microhardness values decreased significantly in the Icon and Permaseal subgroups, while the lowest microhardness values were observed for the Permaseal subgroup, which was significantly different from the Icon subgroup ($p < 0.001$) (Table 3 and Figure 3). The Icon subgroup exhibited significantly higher mean microhardness following the acidic exposure compared to the negative control group (untreated WSLs), while the Permaseal subgroup showed non-significant higher differences. The microhardness values of the Icon and Permaseal subgroups were significantly lower compared to the positive control group (un-demineralized sound enamel surface) (Table 4 and Figure 4).

TABLE 3. Comparison between the microhardness values before and after the acidic exposure for the Icon and Permaseal groups

	Before the acidic exposure	After the acidic exposure	t-value	p-value
Icon resin infiltration group	169.75±12.97	154.95±9.41	-3.79	0.001*
Permaseal composite resin sealant group	165.80±12.53	137.05±7.76	-9.15	<0.00001*
p-value	0.33 Ns	<0.001*		
t-value	0.97	6.55		

*; Significant at p<0.05. Ns; non-significant.

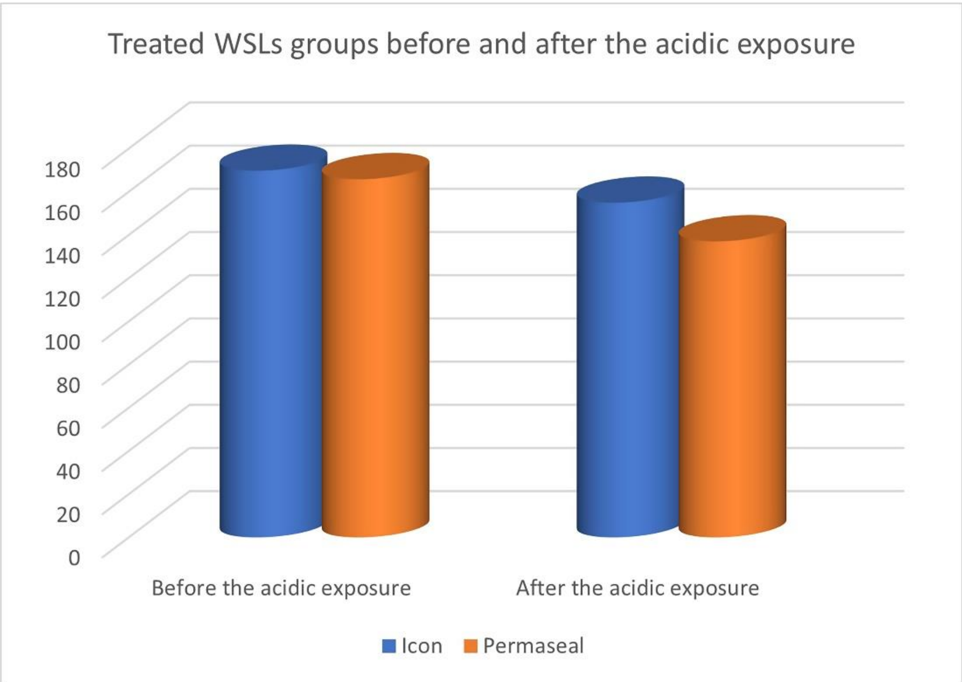

FIGURE 3. Bar chart showing the comparison between the microhardness values before and after the acidic exposure for the Icon and Permaseal groups

TABLE 4. Comparison of microhardness values of positive and negative control groups with those after the acidic exposure for Icon and Permaseal groups

Group	Mean	SD	p-value
Positive control group (sound enamel surface)	181.3^{A}	17.35	0*

Negative control group (untreated WSLs)	129.20^{C}	9.13	
Icon resin infiltration group	154.95^{B}	9.41	
Permaseal composite resin sealant group	137.05^{C}	7.76	

*; Significant at p<0.05.

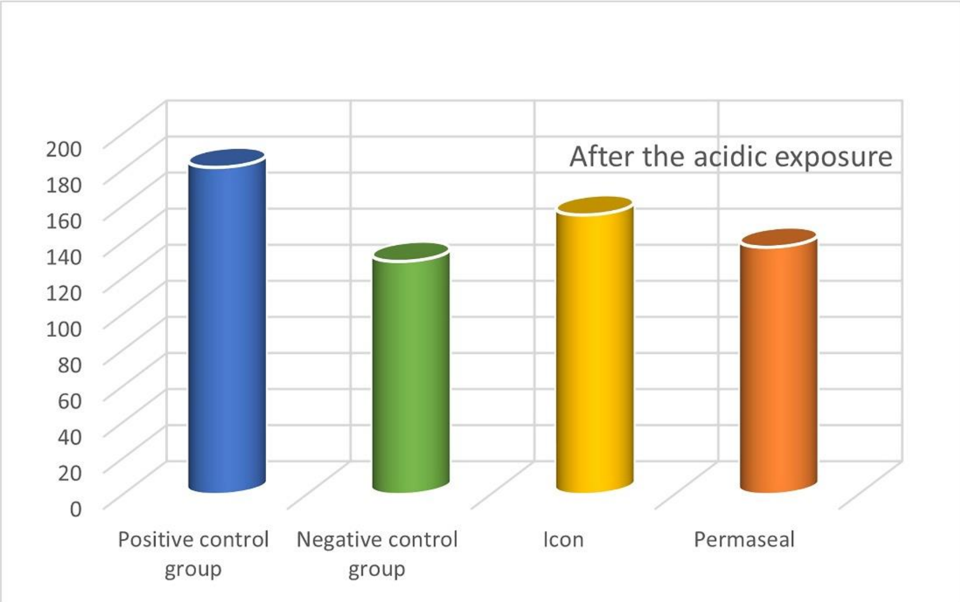

FIGURE 4. Bar chart comparing microhardness values of positive and negative control groups with those of Icon and Permaseal groups following acidic exposure.

5. DISCUSSION

Various risk variables, such as white ethnicity, inadequate oral hygiene, various locations of poor dental hygiene, and beginning therapy at a young age, impact demineralization throughout orthodontic therapy. Demineralization could happen as soon as one month following bracket implantation in people who are predisposed to it. This is because the bacteria in dental plaque dissolves enamel crystals, leading to more pores and resulting in opaque white spots (6).

Clearly, repeated acid exposures lead to insufficient fluoride-based remineralization, and increased fluoride dosage can lead to dental fluorosis and occult caries (30). Over time, the masking of WSLs has evolved, with the most common method being micro-invasive resin infiltration. This technique employs low-viscosity resins to infiltrate lesion pores via capillary action following surface etching, preserving healthy tissue and bridging the gap between preventive and restorative care (24,25). Furthermore, composite sealants are used to fill rough surfaces and penetrate structural micro-defects and marginal gaps with their low viscosity and high wetting ability (33). Therefore, we chose the resin infiltration technique (Icon and composite sealant) to be tested in our study.

Also, microhardness changes in resin-based restorative materials significantly impact restoration longevity, especially in WSLs infiltrated by resin infiltrants. This loss of strength can leave surfaces unprotected, promoting the development of new caries lesions (23). Concerns persist regarding the resistance of resin infiltrants to degradation by various factors in the oral cavity as the PH fluctuation (26).

Suitably, the Vickers surface microhardness test was chosen to assess all samples' microhardness. Microhardness measurement is ideal for materials with fine microstructures, non-homogeneous structures, or cracking proneness, like enamel, offering a simple, nondestructive, and rapid method for demineralization and remineralization studies (34). Additionally, The Vickers surface microhardness test was chosen over the Knoop microhardness test due to its easy and accurate measurement of indent shape (35), allowing for evaluation of resin infiltration's effectiveness in protecting WSLs against cariogenic challenges (23).

Moreover, this study aimed to compare the effect of different resin infiltrations on the microhardness of artificial WSLs before and after acidic exposure (Coca-Cola). This beverage was chosen due to its lower pH (2.5) compared to the tooth's critical pH (5.5), and the increasing consumption among adolescents [(26)].

The present results showed that the microhardness of WSLs treated by Icon and Permaseal increased significantly when compared to the negative control group (untreated WSLs), which may be due to the interaction between monomer and hydroxyapatite in a uniform complex improves mechanical strength [(36)]. The resin fills intercrystalline spaces within the lesion body, sealing it through capillary forces. Polymerization occludes these spaces, slowing or arresting caries progression, thus enhancing the mechanical properties of infiltrated WSLs [(37)]. According to this finding, the first part of the null hypothesis was accepted since the resin infiltrant increased the microhardness of WSLs.

Such outcomes were in line with those of Alagha et al. [(25)], who stated that Icon and ordinary adhesive significantly increased the microhardness compared to the untreated WSLs. In contrast to these findings, de Cerqueira et al. [(24)], found that Icon and other experimental resin infiltration didn’t significantly increase the microhardness of the untreated WSLs.

However, in the current research, the Icon group showed non-significant higher microhardness values than composite sealant (Permaseal). This might be attributed to the difference in the composition of the materials tested [(24)]. As the mechanical properties of low viscosity resin infiltration may be influenced by enamel surface treatment with hydrochloric acid, resin penetration, penetration duration, and infiltrant monomer formulations [(38)]. This finding was in concordance with the study done by Yazkan et al. [(39)], who found that the treated WSLs with Icon had higher microhardness values than ordinary adhesive system.

Indeed, the previous method of infiltrating WSLs with varying penetration coefficients indicated that higher penetration coefficients and ethanol-free resins are more effective for deeper infiltration [(40)]. So, the higher microhardness values of the Icon suggest that infiltrant resin has a better ability to penetrate and fill the voids in WSLs [(41)].

Similarly, Icon has superior penetration ability compared to Permaseal due to its low-viscosity monomer, low molecular weight, and high conversion degree (TEG-DMA) [(42)]. While Bis-GMA monomer (in Permaseal) increases the viscosity and reduces the penetration coefficient [(43)]. Furthermore, resin penetration depth critically influences diffusion barrier formation and the effectiveness of infiltration in halting the progression of caries [(38)]. These explanations were in line with those of Theodory et al. [(28)], who found that the penetration depth of the Icon was deeper than Permaseal.

Also, current results showed that Icon and Permaseal had significantly lower mean microhardness than the un-demineralized sound enamel surface (positive control). This may be due to an insufficiently strong intermolecular bond and the resin not infiltrating all the WSLs. Additionally, the polymerization shrinkage in the resin material may result in unfilled regions of demineralized enamel and the 15% HCL etching before resin application. These factors led to a reduction in the microhardness values [(24)]. These results were agreed with Neres et al. [(23)], who found that the resin-infiltrated WSLs had lower microhardness than the sound enamel.

According to the acidic exposure, our results revealed that the microhardness values decreased significantly in both subgroups after the acidic exposure. This may be due to the resin material infiltrants undergoing degradation and dissolution in an aqueous medium after pH cycling led to the significant drop in microhardness [(44)]. The presence of TEGDMA in the resin infiltrants makes them highly hydrophilic, making them less resistant to deterioration. Additionally, there may have been further dissolution of regions within WSL bodies where the infiltrant was not fully incorporated [(24)]. Based on this outcome, the second part of the null hypothesis was rejected since the microhardness values significantly decreased after the acidic exposure.

Despite this, our results showed that the infiltrated WSLs subgroups (Icon and Permaseal) still had higher mean microhardness following the acidic exposure than the negative control group (untreated WSLs). It may be explained by the resin infiltration involves capillary forces to penetrate and occlude subsurface WSLs, partially or fully replacing missing minerals, enveloping hydroxyapatite crystals, and micromechanically interlocking remaining enamel prisms, making them more resistant to new cariogenic attacks [(45)].

Our findings agreed with those of Alagha et al. [(25)], who found that Icon and ordinary adhesives' microhardness decreased significantly after an acidic exposure but still had higher microhardness compared to untreated WSLs. They explained that the success of resin infiltration depends on its penetration into the lesion's pores and depth, protecting it from further demineralization.

Besides, the infiltrant applications in two layers can compensate for shrinkage and fill porosities in lesion bodies, improving the hardness and reducing mineral loss when subjected to second demineralization [(39)]. Likewise, the penetration of resins into WSLs hindered demineralization, and the application of a second resin layer was crucial for reducing lesion progression, and longer application time improved resin penetration ability [(25)]. Thus, subsurface resin infiltration protects enamel by forming a physical barrier against subsequent acidic challenges [(31)]. On the contrary, our results disagreed with those of Neres et al. [(23)], who reported that resin infiltrant failed to protect enamel against pH cycling.

Appropriately, 11% to 24% of orthodontic patients already develop WSLs when brackets are fixed because of children's and teenagers' increasing beverage intake. To protect the enamel from demineralization and increase patient pleasure with orthodontic therapy, preventive measures like Icon resin infiltration might be required both through and following orthodontic therapy, particularly surrounding wholly brackets [(6)].

This study is limited by its in-vitro design and use of artificial lesions, which may not fully replicate clinical enamel lesions or oral environmental dynamics. While focused on resin infiltration, alternative treatments warrant investigation. Further research should assess long-term material stability under clinical conditions, including salivary exposure and thermal cycling effects

6. CONCLUSIONS

Despite the limitations of an in vitro study, we can conclude that both Icon resin infiltration and Permaseal composite sealant effectively improved the microhardness of artificially induced post-orthodontic WSLs compared to untreated lesions. However, Icon resin infiltration exhibited superior resistance to acidic exposure, maintaining significantly higher microhardness post-acidic challenge than Permaseal. This suggests that Icon creates a more robust and effective barrier against demineralization, offering enhanced protection against further acid attacks.

Clinical Significance:

For individuals undergoing orthodontic therapy, particularly those at high caries risk, early intervention with resin infiltration techniques can be a highly effective minimally invasive strategy to protect and strengthen enamel. Icon resin infiltration is the preferred treatment option for preventing the progression of WSLs and maintaining long-term enamel integrity.

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