

Restorative Dentistry Issue (Removable Prosthodontics, Fixed Prosthodontics, Endodontics, Dental Biomaterials, Operative Dentistry)

## **Effect of Resin Infiltrant versus Universal Adhesive on Microhardness of Acidic Challenged-White Spot Lesions (In-vitro Study)**

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# Effect of Resin Infiltrant Versus Universal Adhesive on Microhardness of Acidic Challenged White Spot Lesions *In-vitro* Study

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## Abstract

**Purpose:** This study investigated the effect of resin infiltrant versus universal adhesive resin on the microhardness of white spot lesions without and with an acidic challenge. **Materials and methods:** A total of 30 human premolars were included. The teeth were cut vertically into two similar halves ( $n = 60$  specimens). The enamel surface of each specimen was partially covered with acid-resistant nail polish, leaving a 4–3 mm as an investigational window. Then, all the specimens were immersed in a demineralizing solution for 72 h, and the tested materials were applied. The specimens were randomly distributed into four groups: A1: untreated sound enamel, A2: demineralized enamel, A3: demineralized enamel + resin infiltrant, and A4: demineralized enamel+the universal adhesive system. For the acidic challenge, the resin infiltrant and universal groups' specimens were further soaked in the same demineralizing solution for another 72 h. The microhardness test was performed once for each specimen in sound and demineralized enamel groups, while for the resin infiltrant and universal adhesive groups, the time of assessment was B1: before the acidic challenge and B2: after the acidic challenge. **Results:** Microhardness results showed that the resin infiltrant group had a significant increase in microhardness compared to the universal adhesive group before and after the acidic challenge ( $P \leq 0.05$ ). **Conclusion:** The ICON resin infiltration system seemed to have a more positive impact on surface microhardness and acidic resistance to the acidic challenge than the All-Bond Universal adhesive.

**Keywords:** Microhardness, Resin infiltrant, Universal adhesive, White spot lesions

## 1. Introduction

White spot lesions (WSLs) are the earliest forms of enamel demineralization that precede cavitation [1]. These lesions are reversible forms of enamel caries. Bacteria colonization and invasion into the lesions may progress into enamel and dentin later on. WSLs are also caused by demineralization after acid attack due to frequent carbohydrate intake, removal of fixed orthodontic appliances, fluorosis, and developmental hypoplasia [2].

Clinically, such lesions reveal an opaque-chalky appearance. This chalky appearance results from mineral loss and increased porosities among the hydroxyapatite crystals [3]. The porosities are later

filled with air or water, whose refractive indices are (1 and 1.33), respectively, compared to (1.62) for the sound enamel. Thus, the differences in refractive indices; lead to light-enamel interactions and dull opaque color. So, continued progression of these lesions without correction increases the risk of cavitation.

Consequently, early intervention will arrest the lesion progression while only minimal invasive approaches were attempted [4]. Remineralization of a non-cavitated carious lesion is a fundamental option in minimally invasive dentistry. Remineralizing agents, like sodium fluoride, casein phosphopeptide amorphous calcium phosphate, and nano-hydroxyapatite regain the physical and biological properties of the lost dental tissues. Furthermore, a modern

Received 10 November 2023; accepted 14 February 2024.  
Available online 10 September 2024

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<https://doi.org/10.58675/2974-4164.1610>

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product was introduced into the market, utilizing the infiltration concept (ICON), which is a resin infiltrant [5].

ICON resin infiltration kit is composed of 15% hydrochloric acid, low-viscosity resin infiltrant, and ethanol. This technique involves the occlusion of acidic diffusive pathways into the depth of the lesion [6]. Besides arresting lesion progression, low-viscosity resin infiltrant benefits teeth' esthetic appearance by a camouflage effect [7]. So, infiltrating the lesion with low-viscous resin improved enamel microhardness and esthetics [8].

Recently, conventional dental adhesives may be considered as substitutes for resin infiltrants, which can reduce the progression of WSLs. Like the resin infiltration technique, adhesives exhibit a deep capillary penetration through the porous lesion following variable conditioning protocols. Universal adhesives, known as multipurpose or multi-mode adhesives, are recent adhesives [9].

Therefore, this *in-vitro* study was conducted to investigate the effect of resin infiltrant versus universal adhesive on the microhardness of WSLs before and after the acidic challenge.

### 1.1. Null hypothesis

There is no difference in the effectiveness between the two tested resin infiltration systems on the surface microhardness of WSLs.

## 2. Materials and methods

### 2.1. Sample size calculation

The sample size was calculated at a significance level of 0.05, with a statistical power of 80% and an effect size of 1.22. The analysis was performed using the G\* Powers software program (version 3.1.9.7) for sample size determination, with a total sample size of 60, subdivided into six groups.

### 2.2. Teeth selection and preparation of specimens

A total of 30 defect and stain-free sound human permanent premolar teeth were utilized after

cleaning and immersion in 0.1% thymol solution for disinfection and preservation. Teeth were rinsed with distilled water and stored in a saline solution containing 10% sodium azide and maintained in this solution at 4 °C till use [10]. The teeth were prepared from the buccal and lingual surfaces of each crown. Two cuts were made using a diamond blade in the cutting machine (ISOMET; 4000 Beulher, Lake Bluff, Illinois, USA) under copious water coolant. The first cut was made in a horizontal direction coronal to the cemento-enamel junction and parallel to the occlusal plane to eliminate the teeth root. The second cut was longitudinal in a mesiodistal direction so that each specimen was divided into two halves. A total of 60 specimens were obtained. Then, each specimen was placed in a prefabricated plastic mold embedded in acrylic resin (Acrostone, Cairo, Egypt) from their pulpal side. An investigational window was created in the middle of the buccal and lingual surfaces of the specimens, with 4 × 3 mm dimensions [11]. The rest of the enamel surface was protected by two coats of acid-resistant nail varnish [12].

### 2.3. Preparation of artificial white spot lesions

The specimens were distributed into four groups: group A1 ( $n = 10$ ) sound enamel, group A2 ( $n = 10$ ) demineralized enamel, group A3 ( $n = 20$ ) demineralized enamel + resin infiltrant (ICON) and group A4 ( $n = 20$ ) demineralized enamel+the universal adhesive resin (Table 1). The last two groups were further subdivided into two subgroups (10 each), according to whether they were subjected to acidic challenge or not: B1 with no acidic challenge and B2 with acidic challenge. Each specimen was immersed separately in a specific jar containing 24 ml of the demineralizing solution for 72 h at 35 °C. Every millimeter square of the exposed enamel received 2 ml of the demineralizing solution [13]. The demineralizing solution was 2.2 mM calcium chloride, 2.2 mM monosodium phosphate, 0.05 M lactic acid solution, and 0.2 pp fluoride ( $\text{CaCl}_2 = 2.2 \text{ mM}$ ;  $\text{NaH}_2\text{PO}_4 = 2.2 \text{ mM}$ ; lactic acid = 0.05 mM; fluoride = 0.2 pp); adjusted with 50% of NaOH to a

Table 1. Description and composition of materials used in the study.

Specifications	Material	Composition
Resin infiltrant	ICON	Icon-etch: 15% hydrochloric acid, pyrogenic silicic acid, water and additives. Icon-dry: 99% Ethanol. Icon-infiltrant: methacrylate-based resin matrix containing Bis-GMA and TEGDMA, initiators, and additives
Hydrochloric acid gel (HCL 15%)	Available in ICON kit	15% hydrochloric acid. Pyrogenic silicic acid. Water. Additives
Adhesive resin bond	All-Bond Universal	Bis-GMA. Phosphate monomer MDP. HEMA. Ethanol. Water
Phosphoric acid etchant	ETCH-37	37% orthophosphoric acid with benzalkonium chloride

pH 4.5. The solution was never changed during the entire demineralization period of each specimen [14]. After the creation of artificial WSLs, each specimen was rinsed with distilled water for 1 min and blot-dried with absorbent paper for 5 s.

#### 2.4. Surface treatment of artificial white spot lesions

For the resin infiltration group, resin infiltrant (ICON) was applied according to the manufacturer's instructions. Fifteen percent hydrochloric acid etchant (Icon-etch) was applied and left for 2 min, rinsed with water for 30 s, and air dried for 30 s. Followed by 99% ethanol (Icon-dry) was applied for 30 s and air dried for 5 s. Then, the Icon-infiltrant first layer was applied for 3 min and light-cured for 40 s. Then, the second layer was applied for 1 min, followed by light-curing for 40 s. Any excess beyond the WSL peripheries was removed before curing. A layer of glycerin was later applied and light-cured for 40 s. Then the infiltrated surfaces were polished according to the manufacturer's instructions. For the universal adhesive group, Bisco All-Bond Universal adhesive was applied according to the manufacturer's instructions for the total-etch technique, with the addition of an intermediary ethanol drying step to standardize the intermediate drying procedure [15]. Thirty-seven phosphoric acid was applied for 20 s, rinsed with water for 10 s, and air dried for 10 s. This was followed by 99.9% absolute ethanol, which was applied for 30 s and air-dried for 5 s. Bisco All-Bond Universal adhesive was then applied and rubbed for 20 s with a micro-brush, gently air dried for 5 s, and light cured for 10 s (Table 1). In between the surface treatments, each specimen was stored in a specific jar containing distilled water at room temperature. The resin infiltration and universal adhesive groups were soaked in the same demineralizing solution used for creating WSLs for 72 h at 35 °C artificially.

#### 2.5. Microhardness assessment

All groups (untreated sound enamel, demineralized enamel, demineralized enamel + resin infiltrant, and demineralized enamel+the universal adhesive system) were subjected to microhardness assessment without acidic challenge. Microhardness assessment in the resin infiltration and universal adhesive groups was performed without and with acidic challenge. The digital display Vickers Microhardness tester (Model HVS-50; Laizhou Huayin Testing Instrument Co. Ltd, Cairo) with a Vickers diamond indenter and a  $\times 20$  objective lens, was used. A load of 200 g was

applied to the surface of the specimens for 15 s. Three indentations were made on the surface of each window. The indentations were placed equidistant, by 0.5 mm, over a circle. The diagonal lengths of the indentations were measured by a built-in scaled microscope, and Vickers values were converted into microhardness values.

#### 2.6. Statistical analysis

Numerical data were explored for normality by checking the distribution of data and using tests of normality (Kolmogorov–Smirnov and Shapiro–Wilk tests). Microhardness data showed normal (parametric) distribution. Accordingly, data was presented as mean and SD values. A one-way analysis of variance test was used to compare groups. Bonferroni's post-hoc test was used for pair-wise comparisons when analysis of variance test is significant. Student's *t*-test was used to compare microhardness values without and with acidic challenge. The significance level was set at *P* value less than or equal to 0.05. Statistical analysis was performed with IBM SPSS Statistics for Windows, Version 26.0. (IBM Corp., Armonk, New York, USA). [16].

### 3. Results

Microhardness assessment of specimens without and with acidic challenge.

Regarding the microhardness assessment without and with an acidic challenge, the highest statistically significant mean microhardness values were recorded in the untreated sound enamel group ( $295.3 \pm 4.9$ ). There was no statistically significant difference between the resin infiltrant group without acidic challenge ( $291.5 \pm 0.2$ ), the resin infiltrant group with an acidic challenge ( $290.6 \pm 0.3$ ), and the universal adhesive group without acidic challenge ( $289.4 \pm 0.3$ ); all showed lower mean microhardness values. Whereas the lowest mean microhardness values were recorded in the universal adhesive group without acidic challenge ( $289.4 \pm 0.3$ ), the universal adhesive group with an acidic challenge ( $287.1 \pm 0.5$ ), and demineralized enamel group ( $287.3 \pm 3.5$ ) (Table 2, Fig. 1).

### 4. Discussion

Enamel carious lesions result from the action of bacterial-secreted acids on the enamel surface. The high amount of acid production from the bacteria in biofilm is associated with poor oral hygiene, which leads to a reduction in pH. This acidic pH causes an off-balance in the process of demineralization and

Table 2. Mean, SD values and results of one-way analysis of variance test for comparing microhardness of all groups.

Groups	Untreated sound enamel (A1)	Demineralized enamel (A2)	Resin infiltrant without acidic challenge (A3, B1)	Resin infiltrant with acidic challenge (A3, B2)	Universal adhesive without acidic challenge (A4, B1)	Universal adhesive with acidic challenge (A4, B2)	P value
Mean	295.3 <sup>A</sup>	287.3 <sup>C</sup>	291.5 <sup>B</sup>	290.6 <sup>B</sup>	289.4 <sup>B</sup>	287.1 <sup>C</sup>	<0.001 <sup>a</sup>
SD	4.9	3.5	0.2	0.3	0.3	0.5	
P value	0.515		3.841		5.177		

Different superscripts indicate statistically significant difference between groups.

<sup>a</sup> Significant at P value less than or equal to 0.05.

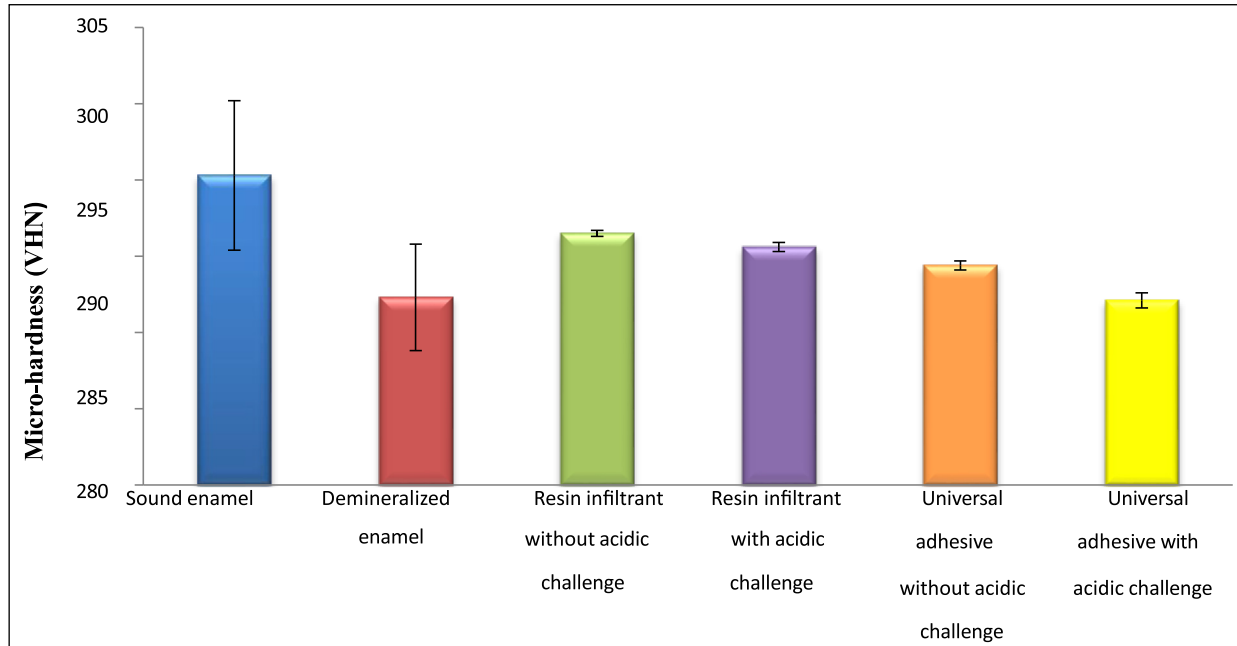


Fig. 1. Bar chart representing mean and SD values of microhardness for all groups.

remineralization of the enamel surface [17]. These lesions are characterized by subsurface demineralization, whereas the enamel surface layer is still intact [18]. WSLs are the first visual alteration of enamel carious lesions [19]. WSLs clinically appear as white opaque zones on the teeth's smooth surfaces. This is due to the subsurface microporosities of the WSLs being loaded with air and water, whose refractive indices differ from that of the sound enamel [7].

The difference in the refractive indices alters light scattering and optical properties, resulting in a chalky white manifestation [19]. Thus, the progression of such lesions should be reversed before their cavitation [20]. Recently, the treatment of WSLs shifted from conventional restorative therapy to more conservative ones, known as minimally invasive techniques [21]. Great attention has been paid to the minimally invasive approach of WSLs with remineralizing agents, such as topical fluoride, CPP-ACP, and fissure sealant. However, these preventive

approaches are only limited to superficial remineralization of WSLs [22].

Infiltrative resins are new materials that provide maximum enamel preservation with immediate results. They have been developed with the purpose of occlusion to the remained microporosities and enhancement to the mechanical strength [23]. The resin infiltration procedure is carried out by using resins that form a diffusion barrier into the WSLs. Thus, after resin infiltration and light curing, subsurface microporosities are occluded with blockage to the acidic diffusive pathways and, accordingly, improve the strength of the remaining enamel [6].

Therefore, this study was performed to investigate the effect of resin infiltrant versus universal adhesive resin on the microhardness of WSLs, without and with an acidic challenge.

ICON infiltration resin is a brand name of a novel product. It displaces the lost minerals in the subsurface porous enamel with a low-viscous light



curable resin. It comes as a kit with three different components. Icon-etch, a syringe filled with 15% hydrochloric acid, Icon-dry, a second syringe in order, contained 99% ethanol and Icon resin infiltrant. The last syringe is composed of a mixture of triethylene glycol dimethacrylate (TEGDMA) and bisphenol A-glycidyl methacrylate (Bis-GMA). ICON resin infiltration showed acceptable outcomes in rehardening the demineralized enamel [24].

Universal adhesives are newly developed types of adhesives. They can be utilized in etch-and-rinse, self-etch, and selective enamel etching modes. All-Bond Universal adhesive resin is composed of Bis-GMA, 10-MDP, 2-hydroxyl ethyl methacrylate (HEMA), ethanol, water, and initiators. This universal adhesive could be used to arrest the progression of the WSLs [25].

Microhardness testing is commonly applied to measure tooth hardness. This procedure is simple and quick, and only a tiny part of the specimen surface is needed for the testing. It is an effective approach for obtaining indirect information about the changes in the mineral content in dental hard tissues. In addition, the test is beneficial in the assessment of the demineralized and treated lesions at various depths of enamel [26].

A resin infiltrating technique was used in this study to cease the WSLs and increase their resistance to further acidic challenge. The difference in composition clarifies the different surface microhardness readings between the two tested resin infiltrants. Fifteen percent hydrochloric acid etchant erodes the surface layer more efficiently than 37% phosphoric acid etchant. Applying longer acid conditioning with ICON (2 min with hydrochloric acid) could have led to deeper resin infiltration than etching with phosphoric acid. The Icon-dry (which contains 99% ethanol) was applied for 30 s before the application of the infiltrant. The addition of ethanol increases the penetration coefficient by lowering the contact angle. Mixtures involving large quantities of HEMA, TEGDMA, and ethanol are associated with high penetration coefficient and satisfactory surface microhardness, and they could be promising tools for fast caries infiltration [27].

The protective capability of the resin also displayed a significant increase in Vickers microhardness when the infiltrant was added in two layers instead of one application. The presence of the resin infiltrant in the subsurface lesion has a protective impact on the enamel; as it acts as a barrier, blocking the pores created by WSLs, enamel then becomes more resistant to new acidic challenges. Consequently, the repeated application of the resin enhanced both surface microhardness and lesion resistance to

demineralization. In addition, the commercially available resin infiltrant has a characteristic organic matrix containing Bis-GMA and a large quantity of TEGDMA. Bis-GMA-based infiltrants are intended to increase the surface microhardness of infiltrated WSLs for their aromatic backbone and stronger molecular structure compared to TEGDMA, thus increasing the polymer hardness. Yet, infiltration with Bis-GMA-containing resins did not lead to increased surface microhardness nor resistance to demineralization relative to resins containing neat TEGDMA. This is due to decreased penetration depth, as Bis-GMA has a higher viscosity than TEGDMA, thus decreasing the resin penetration coefficient [28].

In this study, without acidic challenge, the ICON resin infiltrant group exhibited an increase in the mean value of microhardness compared to the All-Bond Universal adhesive resin group. This could be due to the ICON resin composition being based on an unfilled light-cured low-viscous resin. It is composed of a mixture of TEGDMA and Bis-GMA. The Bis-GMA in the ICON resin infiltrant is known for its high molecular weight and rigid molecular structure. It also contains hydroxyl groups that generate strong hydrogen bonds. These are responsible for the increased microhardness values of the ICON resin infiltrant group [29].

The findings of this study are in line with previous studies [30,31], which concluded that ICON resin infiltrant can be considered a superior treatment option for increasing the surface microhardness of WSLs.

The results of this study also disclosed that the ICON resin infiltrant group showed higher mean microhardness values than the demineralized enamel group but lower mean microhardness values compared to that of the untreated sound enamel group. This was consistent with a previous study [32]; certain areas in the demineralized enamel remained noninfiltrated during the polymerization due to the material shrinkage. Additionally, the polymer chain of the TEGDMA monomer in ICON resin infiltrant may not form optimally and lacks aromatic rings and strong secondary bonds. These factors may contribute to the surface microhardness of the ICON resin infiltrant group not reaching that of the sound enamel group.

Contrariwise, in this study, the All-Bond Universal adhesive resin group demonstrated a decrease in the mean microhardness values. This is due to the presence of HEMA, which has a decreased penetration ability in comparison to the ICON resin infiltrant group. Also, the ethanol content in the universal adhesive resin resulted in insufficient polymerization, giving it liquid consistency. So, this

characteristic hampered its penetration. This agrees with another study [33] that compared the ICON resin infiltrant and two adhesive systems concerning their penetration to the WSLs and concluded that the ICON resin infiltrant showed the highest penetration to the WSLs in comparison to the other two adhesive systems.

With the further acidic challenge, the mean microhardness values of the ICON resin infiltrant decreased but remained higher than the All-Bond Universal adhesive resin group. This is due to, the TEGDMA in the ICON resin infiltrant and enamel hydroxyapatite forming a uniform complex, and the interaction of these crystals improved the resistance to the acidic attack and, thus, slowed the loss of the mineral content in the enamel [15]. The addition of ethanol and TEGDMA to ICON resin infiltrant aids in the reduction of the viscosity and contact angle of the material, thereby increasing the permeability coefficient.

This finding was consistent with another study [34] that concluded ICON resin infiltrant was more resistant than a single bond universal adhesive resin in microhardness upon exposure to the acidic challenge.

However, the results of this study differ from another study [35], which has proved that ICON resin infiltrant did not show resistance to new acidic attacks. These discrepant results may be due to differences in the demineralization protocols for *in-vitro* evaluations. Removal of excess resin infiltrant in the current study during polishing may be another cause. Such polishing may have reduced the thickness of the resin infiltrant and its resistance to acidic attacks as well.

All-Bond Universal adhesive resin group showed the lowest mean microhardness values with an insignificant difference to the demineralized enamel group. Universal adhesive forms a very thick oxygen-inhibited layer that prevents further demineralization. However, this layer was in-homogenous and only a partly polymerized resin layer due to the presence of water. Therefore, the All-Bond Universal adhesive resin-treated WSLs could not resist further acidic attack [36].

According to the results of this study, the null hypothesis is rejected where the ICON resin infiltration system has a more positive effect on the surface microhardness of WSLs over the All-Bond Universal adhesive system.

#### 4.1. Conclusion

Within the limits of this study, the ICON resin infiltration system performed superiorly on the

surface microhardness of the WSLs than the All-Bond Universal adhesive.

#### 4.2. Recommendations

- (1) Further, *in-vivo* studies are recommended to prove the efficacy of the ICON resin infiltration technique.
- (2) Modification to the ICON resin infiltration system formula to seal more microporosities of WSLs.
- (3) Different types of adhesives are to be tested to enhance the surface microhardness of WSLs.

#### Funding

No funding.

#### Ethical approval

This research was performed following international guiding principles, after the approval of the Research Ethical Committee (REC-OP-23-03) of the Faculty of Dental Medicine, Al-Azhar University for Girls.

#### Conflicts of interest

There are no conflicts of interest.

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