

A Comparative Study in Penetration Depth of Resin-based Materials into White Spot Lesion

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Abstract:

Purpose: This in vitro comparative study of penetration depth of resin-based materials: resin infiltrant material, two-steps self-etch resin-based material and solvent free self-etch resin-based material into white spot lesion at different storage times: one day, one week and one month.

Material and Methods: A total number of 60 human posterior teeth (Age range 25-40yr), free from caries extracted for a pathological reason were collected to be used in this study. The buccal and lingual surface for each collected tooth were marked for differentiation, making a total of 120 samples of surfaces, which represented the operational sample size. The 120 samples were randomly divided into four equal main groups (n=30) according to the resin-based materials (M). Group 1 (M1) was assigned as a control group. Group 2 (M2) was restored with Icon. Group 3 (M3) was restored with two step self-etch adhesive system. Group 4 (M4) was restored with solvent-free self-etch adhesive system. Each group was divided into two equal groups (n=15) according to the surface treatment (W). where the first group (W1) was subjected to surface treatment while the second group (W2) was without surface treatment. The samples were further divided into three subgroups (n=5) according to the storage time (S). Subgroup 1 (S1) was stored for one day. Subgroup 2 (S2) was stored for one week. Subgroup 3 (S3) was stored for one month.

Results: The results showed that the highest thickness was recorded in Icon group, followed by the (OptiBondTH) group, then the (Bond-1 SF) group. While the lowest thickness was recorded in the control group. The difference between

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different average thickness was statistically not significant ($p>0.05$). For the without surface treatment groups (W2) the highest average thickness was recorded, while the with surface treatment groups (W1) was recorded the lowest average thickness. The difference between different average thickness was statistically not significant ($P>0.05$). For the Icon group the highest average thickness (S1M2) was recorded on the first day, followed by OptiBondTM XTR (S1M3) group on the first day, while the Bond-1 SF group after one month (S3M4) recorded the lowest average thickness. The difference between different average thickness was statistically not significant ($P>0.05$). For the day one subgroups (S1), the highest average thickness was recorded, followed by the one-week subgroups (S2), while the one-month subgroups (S3) were recorded the lowest average thickness. It was found that the (W1) groups recorded the highest penetration depth values while, the (W2) groups recorded the lowest penetration depth values. The difference between different average penetration depth values was statistically significant ($P<0.05$) as demonstrated by T-test.

Conclusions: Icon show the best thickness value and penetration depth into (WSLs), while solvent-free adhesive system show the worst thickness value and penetration depth into (WSLs). Surface treatment has positive effect on the penetration depth of the resin-based materials, while it's had a negative effect on the resin-based materials thickness. Storage time has a negative effect on the thickness of the resin-based materials.

Keywords: penetration depth, average thickness, resin infiltrant material, White Spot Lesion.

Introduction:

White spot lesions (WSLs) are early signs of demineralization under intact enamel, which may or may not lead to the development of caries. leached out a certain amount of calcium and phosphate ions that may or may not be replaced naturally by the remineralization

process. The causes of (WSLs) may include plaque accumulation particularly along the cervical margins of teeth, inadequate home oral care, and/or consumption of diets rich in sugar those that frequently lower the intraoral PH. (WSLs) may also be seen after removal of orthodontic bands and brackets.⁽¹⁾ The recently introduced alternative therapy is the use of resin-based materials over the dental tissue; which completely fills pores within the tooth, replacing lost tooth structure and stopping caries progression. Resin infiltration system Icon is a new approach that was developed to counteract incipient enamel caries lesions. In contrast to conventional sealants, in which the material adheres to the enamel surface, resin infiltration penetrates into the porous lesion body of enamel's initial carious lesions using a special low-viscosity resin that blocks the diffusion of acids into the lesion, so it blocks the demineralizing effects of cariogenic acids, thereby slowing or arresting the progression of caries.⁽²⁾ Self-etch adhesives contain high concentration of solvents which must be eliminated after completing their function because the residual solvent lead to deterioration of the adhesive interface between tooth structure and composite resin by interfering with resin polymerization. Complete solvent elimination by air drying difficult to achieve, consequently, some residual solvent remains trapped in the adhesive.⁽³⁾ Solvent-free adhesives are hydrophobic, dense and less water sorption and solubility than solvated resin blends materials, so it enhance the tooth adhesion as it free from the solvent residue.⁽⁴⁾ Also, pretreatment of the (WSLs) surface with phosphoric acid etch may show a higher result of penetration coefficient as it allows for more surface area to be coated by resin-based material. This most suite the two step self-etch adhesive system. As the main idea of self-adhesive system is to reduce dentine sensitivity and as long as the (WSLs) are confined in enamel only, so surface treatment may add value to its penetration depth into (WSL) without post-operative sensitivity.⁽⁵⁾ Confocal laser scanning microscopy (CLSM) was used to determine the amount of resin-based material

penetrated inside the (WSLs). Thus, the success of infiltration technique, depends on the efficacy of this low viscosity resin to penetrate up to the depth of the (WSL) and not just mask the lesions.⁽⁶⁾ Although clinical studies have been done earlier. They focused mainly on the clinical success and outcome of the resin. Depth of resin penetration could be a key determining factor for the creation of a diffusion barrier and the success of infiltration. Hence, the aim of the present study is to compare the depth of penetration of a commercially available resin infiltrate system and two bonding agents in (WSLs).⁽⁷⁾ Accordingly, the current study will evaluate the effects of the application of resin-based materials, including resin infiltration, on enamel subjected to (WSLs) challenges and the influence of enamel surface conditioning prior to material application.

Material and Methods: The used materials composition, manufacturer, website and batch number are listed in the table (1)

Table (1): Materials used in the study:

	Brand name	Composition	Manufacturer	Lot Number
1-	Scotchbond™ Universal Etchant	32% phosphoric acid by weight and has a pH of approximately 0.1. fumed silica and a water-soluble polymer	3M ESPE Dental Products,2510, Conway Avenue, St. Paul, MN 55144-1000 USA www.3MESPE.com	6520011690261
2-	Icon	Icon-Etch: Hydrochloric acid, pyrogenic silicic acid, surface-active substances Icon-Dry: 99% ethanol Icon-Infiltrant: Methacrylate-based resin matrix, initiators, additives	DMG Chemisch-Pharmazeutische Fabrik,GmbH Elbgaustraße 248 22547 Hamburg www.dmg-america.com	220401
3-	OptiBond™ XTR two-component. Self-etch universal Adhesive.	Primer: Monomers: Glycerol phosphate dimethacrylate (GPDM) self-etching adhesive monomer and Hydrophilic co-monomers including mono and di-functional methacrylate monomers Solvents: Water, acetone, ethyl alcohol Photo initiator: Camphor quinone (CQ)-based Adhesive: Monomers: Hydrophobic, structural, and cross-linking monomers Solvents: Ethyl alcohol Photo initiator Camphor quinone (CQ)-based Fillers: 0.4-micron barium glass Nano-silica Fluoride: Sodium hexafluorosilicate	Kerr corporation. 1717 west Collins Ave. orange, CA 92867 USA (800) KERR-123 www.Kerrdental.com	6581569 6623732
4-	Bond-1®SF Solvent-Free SE Adhesive	7,7,9(or 7,9,9)-trimethyl-4, 13 dioxo-3,14-dioxa-5, 12 diazahexadecane-1, 16-diyl bismethacrylate 2-hydroxyethyl methacrylate 2,2'-ethylenedioxydiethyl dimethacrylate dimethacrylate diphenyl(2,4,6-trimethylbenzoyl) phosphine oxide	Manufactured by Pentron Clinical 1717 West Collins Ave. Orange, CA 92867 USA www.pentron.com	N03N

Preparation of the samples: The buccal and lingual surface for each collected tooth were marked for differentiation, making a total of 120 samples of surfaces, which represented the operational sample size. The crowns of all teeth were separated from the roots by a diamond-coated band saw under continuous water cooling, then stored in saline solution again until the study.

1. Grouping of the samples for measuring thickness and depth of penetration of the resin based materials: The 120 samples were randomly divided into four equal main groups (n=30) according to the resin-based materials (M). Group 1 (M1) was assigned as a control group. Group 2 (M2) was restored with Icon. Group 3 (M3) was restored with two step self-etch adhesive system. Group 4 (M4) was restored with solvent-free self-etch adhesive system. Each group was divided into two equal groups (n=15) according to the surface treatment (W). where the first group (W1) was subjected to surface treatment while the second group (W2) was without surface treatment. The samples were further divided into three subgroups (n=5) according to the storage time (S). Subgroup 1 (S1) was stored for one day. Subgroup 2 (S2) was stored for one week. Subgroup 3 (S3) was stored for one month

2. Fabrications of the molds: A specially fabricated circle plastic mold of internal diameter 10 mm and 20 mm in height was fabricated. A separating medium was used to coat the internal surface of the mold. The mold was filled with self-curing acrylic resin, the base of the mold rested on a glass slab in order to obtain a flat smooth surface base. Buccal and lingual surfaces of the teeth was embedded horizontal in the mold while the acrylic resin still in the dough stage to be flashed with the level of the plastic mold.

3. Creation of enamel white spot lesion (WSL): The buccal and lingual surface of all samples were subjected to a short-term acidic exposure by application of phosphoric acid H₃PO₄ 37% to the surfaces for one minute to create artificial (WSLs).

4. Surface treatment:

A. Subgroup (W1):

Fifteen samples were surface treated with phosphoric acid H₃PO₄ 37% for thirty second and rinsed with water for two seconds then air dried with oil-free air spray. Resin based material applied, according to manufacturer instructions and immersed in artificial saliva which was changed daily.

B. II. Subgroup (W2):

Fifteen samples were treated with resin-based material according to manufacturer instructions without any prior surface treatment, then immersed in artificial saliva which was changed daily

5. Application of the resin-based materials: Each resin-based material was applied according to the manufacturer instructions as shown below:

Icon application procedure: Icon-Etch (hydrochloric acid) was applied and allowed to sit for two minutes then rinsed off with air water spray for at least thirty seconds, then samples were dried gently with oil free air. Icon-Dry was applied to the lesion site and left for thirty seconds, followed by gently air drying. Icon-Infiltrant was applied to the etched surface and allowed to sit for three minutes, then light-cured for forty seconds using Elipar Light cure unit.

(OptiBond™ XTR) application procedure: Apply (OptiBond™ XTR) Primer to the enamel surface using the disposable applicator brush. Scrub the surface with brushing motion for 20 seconds. Then air thin for 5 seconds with medium air pressure. Shake light brushing motion for 15 seconds. Again, air thin for 5 seconds. Then light cure using Elipar Light cure unit. for 10 seconds.

Bond-1 SF application procedure: Bond-1 SF was being applied with the flocked tip and rubbed for 20 seconds. Light cure the bonding agent for 10 seconds using Elipar Light cure unit*.

6. Storage of the Specimens: All samples were stored in artificial saliva at 37° in an incubator with 100% humidity at different storage times (one day, one week, and one month).

7. Measurement of samples:

I-Measurements of resin-based materials thickness using profilometric analysis*:

A Total number of 120 buccal and lingual surface were divided randomly into two groups 60 samples each. Acid etch (surface treatment) was applied on the buccal or lingual surface of one group, then mark was made on the model surface corresponding to the etched surface either its buccal or lingual to facilitate differentiation between etched (treated surface W1) and unetched (untreated surface W2). Profilometric analysis was performed at the treated and untreated surfaces as a baseline measurement. Adequate measures were made to ensure that only one half of the total number of the samples was treated with acid etches, while the other half was left without surface treatment after (WSL) creation and before application of resin-based materials. Profilometric analysis was performed again at the same surfaces used for the baseline measurements. The resin-based materials were applied on the buccal and lingual enamel surfaces. Profilometric analysis was performed again at the same sites used for the baseline measurements. Then, the samples were stored in artificial saliva for one day, one week, and one month. Then evaluated with the profilometer at the same surfaces used for the baseline measurements after each storage period to determine the resin-based material thickness after application and material loss. Using specific software by calculating the average thickness of the materials and the depth of the lesion surface relative to the baseline surface profiles, respectively

II-Measurement of penetration depth of resin-based materials into (WSL):

Preparation of resin material for (CLSM)

Analysis: A 0.05 mg/ml ethanolic solution of tetramethyl rhodamine isothiocyanate*, was

used to label the resin-based materials under study by adding 0.02 ml of this solution in 0.5 ml. Resin penetration was observed using (CLSM) **, which was equipped with four solid state laser from 488 to 635 nm. The samples were observed using a 403 objective in fluorescence (wavelength $\lambda=532$ nm) and reflection (wavelength $\lambda=488$ nm) mode.

Resin penetration depth measurement using (CLSM):

Two teeth were selected randomly from each (W1) and (W2) groups. Then picture captured for the samples by (CLSM) were assessed. For measuring the penetration depth of the resin-based materials and comparing each result with other groups.

Results: The results showed that the highest thickness was recorded in Icon group, followed by the (OptiBondTH) group, then the (Bond-1 SF) group. While the lowest thickness was recorded in the control group. The difference between different average thickness was statistically not significant ($p>0.05$). For the without surface treatment groups (W2) the highest average thickness was recorded, while the with surface treatment groups (W1) was recorded the lowest average thickness. The difference between different average thickness was statistically not significant ($P>0.05$). For the Icon group the highest average thickness (S1M2) was recorded on the first day, followed by OptiBondTH XTR (S1M3) group on the first day, while the Bond-1 SF group after one month (S3M4) recorded the lowest average thickness. The difference between different average thickness was statistically not significant ($P>0.05$). For the day one subgroups (S1), the highest average thickness was recorded, followed by the one-week subgroups (S2), while the one-month subgroups (S3) were recorded the lowest average thickness. It was found that the (W1) groups recorded the highest penetration depth values while, the (W2) groups recorded the lowest penetration depth values. The difference between different average penetration depth values was statistically significant ($P<0.05$) as demonstrated by T-test.

Discussion:

(WSLs) are early signs of demineralization under intact enamel, which may or may not lead to the development of caries. (WSLs) occur when the pathogenic bacteria have breached the enamel layer and organic acids produced by the bacteria have leached out a certain amount of calcium and phosphate ions that may or may not be replaced naturally by the remineralization process.(8) The progression of (WSLs) can be slowed or even arrested by non-operative measures that influence etiologic factors such as maintaining oral hygiene and use of remineralizing agents such as topical fluorides and casein phospho peptide-amorphous calcium phosphate.(9) according to the classification of the WSLs. Group 1: Visible WSLs without surface disruption and Group 2: WSLs showed a roughened surface but not requiring restoration. Three successive photographs were taken for every patient; immediately after bracket removal, 1 week after oral hygiene measures and after Icon material application. The JPEG images were imported into image analysis software (Image J version 1.33u for Windows XP, US National Institutes of Health Alternative therapy is the use of resin-based materials over the dental tissue. which completely fills pores within the tooth, replacing lost tooth structure and stopping caries progression.(10) a caries infiltrant might be also used for enamel conditioning prior to composite insertion. This study aimed to analyse the shear bond strength of a flowable composite to sound and demineralised enamel pretreated with a caries infiltrant, a conventional adhesive or a combination of both. Methods: Flattened bovine enamel specimens (n = 120 Icon is a new approach that was developed to counteract incipient enamel caries lesions. In contrast to conventional sealants, in which the material adheres to the enamel surface, resin infiltration penetrates into the porous lesion body of enamel's initial carious lesions using a special low-viscosity resin that blocks the diffusion of acids into the lesion, so it blocks the demineralizing effects of cariogenic acids, thereby slowing or arresting the progression of caries.(11) Solvent-free adhesives are

hydrophobic, dense and less water sorption and solubility than solvated resin blends materials, so it enhance the tooth adhesion as it free from the solvent residue.(4) Also, pretreatment of the (WSLs) surface with phosphoric acid etch may show a higher result of penetration coefficient as it allows for more surface area to be coated by resin-based material. This most suite the two step self-etch adhesive system. As the main idea of self-adhesive system is to reduce dentine sensitivity and as long as the (WSLs) are confined in enamel only, so surface treatment may add value to its penetration depth into (WSL) without post-operative sensitivity. Determining the thickness of the resin-based material over the (WSL) and the depth of the (WSL) let us know the amount of surface loss from the resin based materials or from the enamel structure and feedback about the progression of (WSL) in the samples Profilometer and Optical Profilometer are typically used to measure the surface hardness but it can be used to measure the thickness indirectly, through special software which interpret scanned tomography images of the surface.(5) In the current study, we made benefits from these advantage of the images of the profilometer in determining the depth of the (WSL) as well as the thickness of the applied resin based materials. The images and measurements helped to assess precisely the changes in depth of (WSL) and depreciation of the applied materials along study time.(12) Also determining the penetration depth of the resin-based materials into the (WSL) let us know the efficacy of the resin-based materials to penetrate the enamel structure and establish a successful adhesion bond with it. In this respect, Confocal laser scanning microscope (CLSM) and electron scanning microscope are the recent introduced microscopes respectively that allow explore the microscopic structure of the sample and distinguish the different layers within it. The (CLSM) has become a popular tool for life sciences researchers, primarily because of its ability to remove blur from outside of the focal plane of the image.(6)

The current study revealed that: the Icon group recorded the highest vertical-height

mean value which means the lowest surface loss. This may be due to: Icon prevents progression of the enamel (WSLs) through infiltration in the micro porosities resulting in occluding it and increasing the surface thickness and hardness.

Which is supported by (Belli et al, in 2011) (13) and to assess their ultramorphology. Methods: Flat enamel surfaces from freshly extracted bovine teeth were polished and immersed in a Buskes demineralising solution for 30 days to create incipient caries-like lesions (white spots who found that: The vertical surface loss values of the (WSLs) treated with the resin infiltration showed the same result of the original enamel, indicating that this material might be suitable for the treatment of enamel WSL. This is also in agreement with (Oliveira et al, in 2015) (5) who found that: The Icon was able to protect the enamel, even after exposure to erosive challenge, the thickness of the Icon that covered the enamel surface was nearly the same, regardless of enamel conditioning. This is also in agreement with (Senestraro et al, in 2013) (14) who found that: Resin infiltration significantly improves the clinical appearance of WSLs, with stable results seen eight weeks after treatment. Practically resin infiltration, a minimally invasive restorative treatment, was shown to be effective for WSLs that formed during orthodontic treatment. However, this disagrees with (Wolfgang H et al, in 2014) (15) who found that: Resin infiltration has some limitations of its technique that are surface conditioning, porosity of the lesion and the extreme hydrophobicity of the resin. This controversy may be due to the using of different manufacture lot or even storage media that couldn't match manufacture storage instruction. Perhaps the test and research that have been done on the Icon gives approximately the same result without mention that limitations. Also it disagree with (Elhiny OA et al, in 2016) (16) who concluded that the Icon surface pretreatment sealed the enamel porosities in the infiltrated enamel but not improves the surface hardness compared to sound enamel. However, the (OptiBond™ XTR) group recorded the mean value of the surface loss

lower than solvent free adhesive group. This may be due to: The chemical composition and stability of the material itself. That could lead to some sort of surface loss from the material. This agrees with (Rahiotis C et al, 2011) (13) and to assess their ultramorphology. Methods: Flat enamel surfaces from freshly extracted bovine teeth were polished and immersed in a Buskes demineralising solution for 30 days to create incipient caries-like lesions (white spots who found that: when the Icon versus the two-step adhesive system applied over the caries lesion was compared with the original enamel, non-significant differences in vertical surface loss were measured. However, the Icon material showed surface and morphological aspects that pointed to improved surface stability and infiltration quality Thus, a thicker layer of Icon might be beneficial for surface loss resistance. Also, in this study, the solvent free adhesive group recorded the highest mean value of vertical tooth loss than the other groups. This may be due to mainly adhesive failures with partial cohesive failures in the adhesive resin. This is in agreement with (Koumpia et al, 2014) (17) who found that: eliminating solvents from self-etch adhesive systems may decrease the bonding strength and cause adhesive failures with partial cohesive failures.

The current study revealed that: The highest penetration depth was recorded in Icon group with surface treatment (W1M2). While, the lowest penetration was observed in (Bond-1 SF) group without surface treatment (W2M3) with statistically significant difference in the penetration depth ($P < 0.05$). This may be due to: The Icon is basically composed with the monomer triethylene glycol dimethacrylate (TEGDMA) and resinous sealant consists of TEGDMA and bisphenol glycidyl methacrylate (BisGMA) monomers. The TEGDMA based materials show greater penetration due to their low viscosity, high degree of conversion, and high penetration coefficient. This is in agreement with (Ionta et al, in 2016) ⁽⁸⁾ who found that: The Icon showed greater depth and homogeneity of penetration than the other materials, followed by the resinous sealant. However, the (OptiBond™ XTR)

group recorded the mean value of the depth of penetration less than Icon group but higher than solvent free adhesive group. This may be due to: the presence of the hydroxyethyl methacrylate (HEMA) which has less penetration ability in comparison with Icon group. Also, in this study: The solvent free adhesive group recorded least penetration depth mean value. This may be due to: mainly the absence of solvent which has a significant drop in the penetration coefficient. This is in agreement with **(Koumpia et al, 2014)**⁽¹⁷⁾ who found that: eliminating solvents from self-etch adhesive systems may decrease penetration depth and cause adhesive failures with partial cohesive failures.

The current study revealed that: All groups that have been treated with surface conditioning revealed the highest mean value of penetration. This may be due to: the more surface area and pores plus the dissolution of the prismatic layer of the enamel.

This in agreement with **(Ionta et al, in 2016)**⁽⁸⁾ who found that: The etching with phosphoric acid improves the mechanical retention of dental materials by promoting the dissolution of prismatic and inter-prismatic enamel and creating irregularities in the enamel “prisms” in which the resin-based material can flow. This also, in agreement with **(Pashley et al, 2011)**⁽¹⁸⁾ who found that: Etch-and-rinse adhesives produce higher resin-dentin bonds that are more durable than most 1 and 2-step adhesives.

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