Effect of Different Bleaching Methods and Storage Periods on Nanoleakage

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Abstract: Objectives: To assess the performance of two bonding agents when subjected to different bleaching mechanisms and stored for three storage periods in preventing nanoleakage of class V restorations. **Methods:** BOND 1 SF (B1) [solvent-free self-etching adhesive system, Pentron Clinical] and Xeno V (XV) [self-etching adhesive system, Dentsply] used with ESTHET.X HD resin composite (visible light-cured composite material, Dentsply). Class V cavities were prepared (4mm length x2mm width x2mm depth) on buccal surfaces of 120 sound human upper centrals. Cavities divided into two groups (n=60) according to the adhesive used. Each group subdivided into two subgroups (n=30) according to the used bleaching systems; Crest 3D White Whitestrips Advanced Vivid (bleaching strips) and Colgate Visible White 9% Mint (home bleaching gel). Each subgroup was stored in artificial saliva for three different periods (n=10; 1) stored for 24hours, 2) stored for 3months and 3) stored for 6months. Teeth then coated with nail polish up to 1mm from the interface, immersed in 50% silver nitrate solution for 24h and tested for nanoleakage using Quanta Environmental SEM and EDAX. Data statistically-analyzed using three-way ANOVA and Tukey's post-hoc tests ($P \leq 0.05$). **Results:** XV showed significantly higher mean percentages of silver penetration (3.75) than B1 (2.89). Colgate gel (3.72) showed significantly higher percentages of silver penetration than crest strips (2.74). Whereas, there was a statistically significant increase in mean percentages of silver penetration by time. **Conclusions:** under the test conditions, BOND 1 SF provided better sealing ability. Meanwhile, the tested home bleaching gel increased nanoleakage for both tested adhesives.
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1. Introduction:

Nanoleakage which is a leakage pattern occurring within the nanometer-sized spaces formed within the hybrid layer[1], is an important indicator for the material's sealability. The stability and durability of the marginal seal are of major significance for the longevity of resin composite restorations. [2]Hence, the absence of nanoleakage is as an important factor in preventing post restorative hypersensitivity, secondary caries and pulpal damage.

In the recent years, growing efforts were made to simplify and shorten bonding procedures [3], yet retain the effectiveness of dentin adhesives. Thus, self-etch adhesives were developed. This 'No rinse technique' not only eliminates operator variables but also lessens clinical operating time. Recently, Solvent-free self-etch adhesive has been introduced with assertions of performing an interactive bond between the minerals of the tooth structure and the resins of the bonding agent, without the use of acetone, water, or alcohol, thus, providing a superior bond to both dentin and enamel.

Tooth bleaching is an important part of esthetic dentistry. It allows patients to lighten the color of their teeth safely. The recent developments in the bleaching agents increased the interest among both dentists and the public. [4] However, bleaching may have different effects on restorations.[5,6] Some authors have revealed negligible effect on the surface of composite restorations. [7]Whereas, other authors found that bleaching slightly increased the surface roughness of resin composites. [8] This in turn can affect the sealing ability of composite restoration.

It has been suggested that the oral environment is likely to cause more pronounced filler degradation than indicated by storage in distilled water. [9] Consequently, storage of resin composite restoration in artificial saliva can affect the sealing performance of these restorations which in turn may result in increased nanoleakage.

This study evaluated the nanoleakage of two self-etch adhesives after exposure to different bleaching methods and storage periods. The null hypotheses tested were: (1) the bleaching methods have similar influence on nanoleakage; (2) nanoleakage is similar for both tested adhesives; and (3) the storage periods do influence the nanoleakage of the tested adhesives.

2. Materials and Methods:

I. Preparation of the cavities:

120 sound extracted human premolars were used in this study. Teeth were cleaned, examined, sterilized using chloramine T solution and stored refrigerated. They were used within a maximum of one month from their extraction. Standardized wedge-shaped Class V cavity was prepared at the cementoenamel junction on

the buccal surface of each tooth. Half of the prepared cavity was above the cementoenamel junction and the other half below it.[10] The prepared cavities were measured 4 mm occlusogingivally, 2 mm mesiodistally and 2 mm in depth. The outline of the cavities was standardized using a stainless steel matrix band into which a window representing the selected length and width was cut into its middle.

Specimen in the present study involved the preparation of Class V cavities because it involves different dental hard structures, enamel, dentin, and cementum. Also, This cavity configuration represents high stress due to higher C-factor.[11,12] Lastly, preparation and restoration of Class-V lesions is minimal and relatively easy, thereby reducing practitioner variability.[13]

II. Grouping of the specimens:

Cavities divided into two groups (n=60) according to the tested adhesive. Materials' composition and manufacturers are shown in table (1). Each group subdivided into two subgroups (n=30) according to the used bleaching systems (Table 2).

BOND 1 SF [solvent-free self-etching adhesive system, Pentron Clinical] and Xeno V [self-etching adhesive system, Dentsply Caulk, Milford, DE,USA] were used to treat the cavity walls of groups I and II respectively according to manufacturer's instructions. The treated cavities were then filled with ESTHET.X HD resin composite (visible light-cured composite material, Dentsply Caulk, Milford, DE,USA) using oblique incremental technique. After restoration of the cavities, each group was further divided into two subgroups (n=30):

1. bleached using Crest 3D White Whitestrips Advanced Vivid (home bleaching strips) (Proctor and Gamble, USA) for 30minutes once a day for 14days,
2. bleached using Colgate Visible White 9% Mint (home bleaching gel) (Colgate Palmolive company, USA) for 30minutes once a day for 14days.

Each subgroup stored after bleaching in artificial saliva and was further subdivided into 3 classes according to the storage periods (n=10); 1) stored for 24 hours, 2) stored for 3 months and 3) stored for 6 months.

III. Storage of the specimens:

The specimens of each class were stored in freshly prepared artificial saliva for the assigned period of time. The composition of the artificial saliva was as follows: 2.0m*M* Ca^{2+}, 1.2m*M* phosphate, 130m*M* KCI and 60m*M* Tris (pH 7.0). The artificial saliva solution was changed daily.

IV. Nanoleakage Assessment:

In order to assess nanoleakage, the root apices of each tooth were sealed with sticky wax and the entire tooth, except for the restoration and 1mm apart from the restoration margins, was coated with two layers of nail varnish. Teeth were then immersed in 50% freshly prepared silver nitrate solution for 24 hours in light-proof container in total darkness, rinsed under running water for 5 minutes then immersed in a photo developing solution for 8 hours while being exposed to a fluorescent light in order to reduce the silver ions to metallic silver. Finally, the teeth were rinsed under running water for 5 minutes to remove the photo developing solution. Each tooth was sectioned longitudinally in a bucco-lingual direction through the center of the restoration and prepared to be examined under electron microscopy.

In this study, nanoleakage was tested by Quanta Environmental Scanning Electron Microscope (QESEM). The QESEM is an analytical tool that provides exceptional depth of field and minimal specimen preparation since it allows examination of the specimens without being coated whether with gold or carbon as in other scanning electron microscopes. EDAX (Electron Dispersive Analytical X-ray) analysis was also carried out in parallel to identify the existence of metallic silver particles. The use of SEM in combination with EDAX had the ability to present both distinct images and sensitive quantification of silver ion penetration, as it permits analysis for the element composition of the scanned square area. This provides accurate identification for the presence or absence of metallic silver particles along the adhesive tooth / restoration interface while eliminating false negative and false positive results. Scanning and EDAX quantification were performed at three points along gingival tooth/restoration interface of each specimen. It was performed at the middle of the tooth/restoration interface, midway between the middle of the tooth/restoration interface and the cavity margin, and near the cavity margin.

For each specimen the readings of the percentage of silver deposition at the three examined points of the gingival restoration interface were summed and divided by three to give the mean of the percentage of silver deposition along the gingival margin.

V. Statistical analysis

The mean of the percentage of silver deposition along the gingival margins was calculated. Means and standard deviations of the percentages of silver penetration data then were calculated for each group and statistically analyzed using IBM SPSS Statistics Version 20 (SPSS Inc., Chicago, IL, USA). Repeated measures Analysis of Variance (ANOVA) was used in testing significance for the effect of adhesive, cyclic loading and their interactions on nanoleakage. Tukey's post-hoc test was used for pair-wise comparison between the mean values when ANOVA test is significant. The significance level was set at $P \leq$

0.05. Statistical analysis was performed with IBM SPSS Statistics Version 20.

Table (1): Manufacturers, manufacturers' instructions and compositions of the used restorative materials

Material	Principal components	Manufacturer
BOND 1 SF (solvent-free self-etching adhesive system)	**The resin matrix:** UDMA, TGDMA, HEMA, 4-META and photocuring system. **The filler:** Silane treated barium glass, silica (amorphous).	Pentron Clinical, USA
Xeno V (self-etching adhesive system)	Bifunctional acrylate, acidic acrylate functionalized phosphoric ester, acrylic acid, water, tertiary butanol, initiator, stabilizer.	Dentsply, Caulk, Milford, DE, USA

Table 2: Bleaching Materials

Material	Manufacturer	Composition
Crest 3D Whitestrips	Crest, Proctor & Gamble, USA	10% hydrogen peroxide
Colgate Visible White Chairside	Colgate Palmolive Company, USA	9% w/w hydrogen peroxide

3. Results:

The results showed that adhesive, bleaching technique, storage time and the interaction between the three variables had a statistically significant effect on mean nanoleakage (Tables 3 and 4).

Effect of adhesive:

Xeno V showed statistically significantly higher mean nanoleakage values than BOND 1 SF, as shown in table (5).

Table (3): Descriptive statistics for nanoleakage values ANOVA results:

Adhesive	Bleaching technique	Storage time	Mean	±SD
BOND 1 SF	Crest 3D Whitestrips	24 hours	0.55	0.19
		3 months	1.02	0.27
		6 months	5.97	0.43
	Colgate Visible White Chairside	24 hours	0.95	0.37
		3 months	2.13	0.34
		6 months	6.73	0.46
Xeno V	Crest 3D Whitestrips	24 hours	0.77	0.24
		3 months	1.38	0.47
		6 months	6.77	0.66
	Colgate Visible White Chairside	24 hours	1.46	0.48
		3 months	2.47	0.69
		6 months	8.58	0.79

Table (4): Regression model results for the effect of different variables on nanoleakage

Source	Type III Sum of Squares	df	Mean Square	*F*-value	*P-value*
Adhesive	7	1	7	29.8	<0.001*
Bleaching techniques	14.3	1	14.3	61.2	<0.001*
Storage time	435.9	2	217.9	934.8	<0.001*
Adhesive x Bleaching technique x Storage time	8.8	2	4.4	17.5	<0.001*

df: degrees of freedom,: Significant at P ≤ 0.05*

Table (5): Comparison between nanoleakage values of the two tested adhesive

BOND 1 SF		Xeno V		*P*-value
Mean	±SD	Mean	±SD	
2.89	1.56	3.57	2.09	<0.001*

**: Significant at P ≤ 0.05*

Effect of bleaching technique:

Colgate Visible White Chairside showed statistically significantly higher mean nanoleakage values than Crest 3D Whitestrips, as shown in table (6).

Effect of storage time:

The statistically significantly highest mean nanoleakage value was found after 6 months. This was followed by 3 months storage period. The statistically significantly lowest mean nanoleakage was found after 24 hours, as shown in table (7).

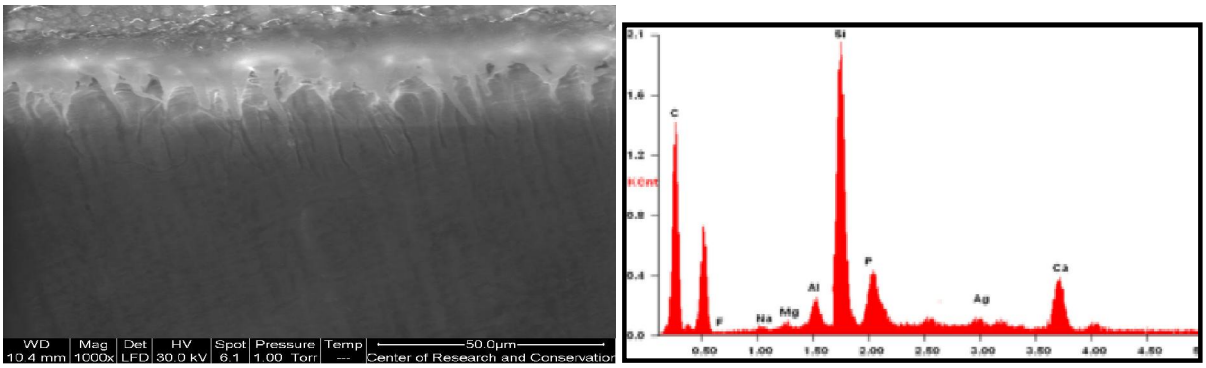

Figure (1): Scanning electron micrograph and its corresponding EDAX spectrum curve for one point at the gingival tooth/restoration interface representing Bond 1adhesive that was bleached with Crest strips.

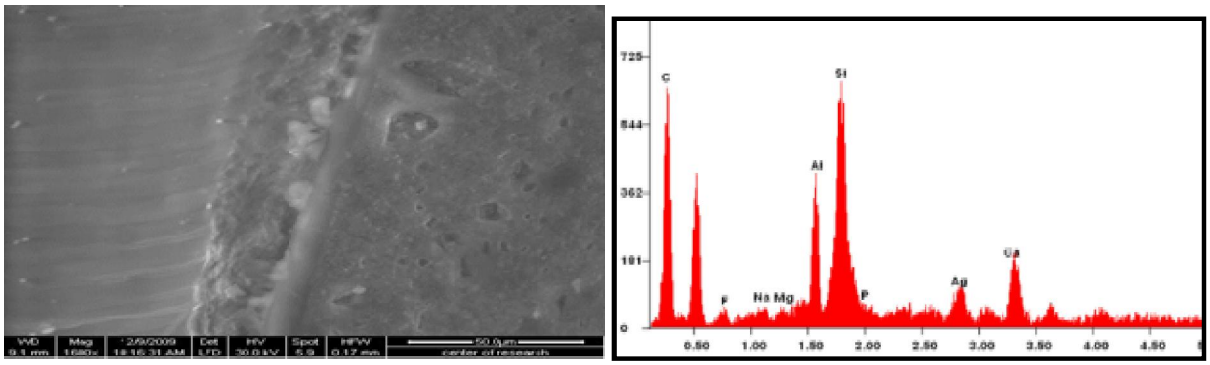

Figure (2): Scanning eectron micrograph and its corresponding EDAX spectrum curve for one point at the gingival tooth/restoration interface representing Xeno V adhesive that was bleached with Crest strips.

Table (6): Comparison between nanoleakage values of the two bleaching techniques

Crest 3D Whitestrips		Colgate Visible White Chairside		*P*-value
Mean	±SD	Mean	±SD	
2.74	1.66	3.72	2.97	<0.001*

**: Significant at P ≤ 0.05*

Table (7): Comparison between nanoleakage values at different storage times

24 hours		3 months		6 months		*P*-value
Mean	SD	Mean	SD	Mean	SD	
0.93 [c]	0.46	1.75 [b]	0.73	7.01 [a]	1.13	<0.001*

**: Significant at P ≤ 0.05, Different letters are statistically significantly different*

Effect of different interactions:

Xeno V with Colgate Visible White Chairside after 6 months showed the statistically significantly highest mean nanoleakage value. BOND 1 SF with Crest 3D Whitestrips after 24 hours showed the statistically significantly lowest mean nanoleakage, as shown in figure (3).

4. Discussion:

The long term durability of adhesive resins is of critical importance to the longevity of bonded restorations. Although high early sealability of current adhesive systems to dentin have been reported, the durability of the adhesive bond is still one of the areas of current interest in adhesive dentistry. [14] This study was conducted to evaluate the sealability of solvent-free self-etching adhesive system compared to that of solvent containing self-etching adhesive system through examining the nanoleakage of Class V composite restorations after bleaching using two different techniques and storage in artificial saliva for three different periods.

The solvent-free adhesive is introduced recently to the market for further simplification of the direct restorative procedures. Bond-1SF solvent-free self etch adhesive does not have any of the conventional

solvents (water, ethanol or acetone) in its chemical composition.[15] Although the absence of the solvent in the composition of the adhesive results in a viscous adhesive necessitating perfect rubbing during its application but still it leads to a thicker adhesive layer. This will likely increase the hydrophilic content in Bond-1SF in comparison to solvent containing adhesive.

In the present study, it was found that, BOND 1 SF (solvent-free adhesive) provided better sealing ability, since Xeno V (solvent containing self-etching adhesive) showed statistically significantly higher mean nanoleakage values. This can be explained by adequate penetration of the adhesive within the dentin due to its increased hydrophilicity in addition to the perfect rubbing of the adhesive during its application.

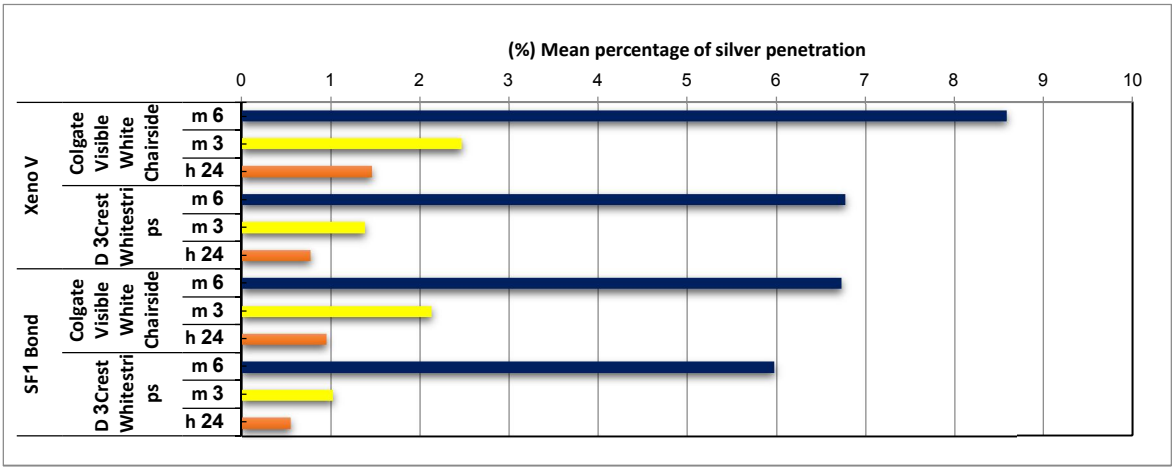

Figure (3): Bar chart representing comparison between mean nanoleakage values of different interactions

However, the result of this study was in contradiction with many studies which evaluated the effectiveness of bonding of self-etching adhesives to enamel and dentin, and concluded that solvent containing self-etching adhesive systems recorded a higher shear bond strength than solvent free self-etching adhesive system. [15,16] Similarly, Shirban *et al.,*[17] found that, the solvent free adhesive B-1SF underperforms as compared to solvent containing adhesives as the control gold standard.Also our result was in contradiction with El Sayed *et al.,* who concluded that, solvent containing self etching adhesive system showed better bonding after thermocycling than the solvent free to dentin. [18]

These authors referred the underperformance of the solvent free adhesive to the fact that it showed thicker adhesive layer and these adhesives behave as semi permeable membranes allowing more fluids to pass through in comparison to solvent containing adhesive, which seems to lead to lower bond strength.

In the present study, Colgate Visible White Chairside (bleaching gel) showed statistically significantly higher mean nanoleakage values than Crest 3D Whitestrips (bleaching strips). However, both bleaching techniques showed nanoleakage at the tooth/restoration interface. This result was in agreement with Ulukapi *et al.,* who suggested that both pre and post-operative bleaching can increase microleakage scores of composite restorations margins.[19] Also, Mortazavi *et al.,* concluded that, bleaching gel has an adverse effect on marginal seal of dentinal walls of existent composite resin restorations. [20] Meanwhile, de Freitas *et al.,* found that, the exposure of dentin to bleaching agents reduces microhardness values. [21] This is due to the fact that using either hydrogen peroxide or carbamide peroxide may result in morphological changes that could reduce the bond between resin restorations and dentin. [22]

On the other hand, the results of Klukowska *et al.,* [23], was in contradiction with the results of the present study. They explored the effects of different concentrations of hydrogen peroxide and carbamide peroxide agents on the enamel margin microleakage of composite restorations. They found that, bleaching agents could not increase the microleakage scores of Filtek Z250 bonded with Scotch bond.

Concern remains regarding the relationship between nanoleakage and long-term storage period since degradation of the resin-dentin interface from hydrolytic attack may slowly occur through nanoleakage pathways. As this nanoleakage pathways may allow fluid penetration along the interface, which may result in a hydrolytic breakdown of either the adhesive resin or collagen within the hybrid layer, it may compromise the stability of the resin dentin bond. [24-28]

The results of this study revealed that, the statistically significantly highest mean nanoleakage value was found after 6 months. This means that, nanoleakage increased by aging. These results are in

accordance with the results found by Okuda *et al.* They revealed that, nanoleakage increased over time within the resin/dentin interface. [29]Also, Frankenberger *et al.,* found that, water uptake in the resin-dentin interface affects the durability of the resin-dentin bond over time. [30]

Since both tested bonding systems contained a hydrophilic constituent, there would be rapid absorption of water.[31,32] This water absorption accounts for increased extension of porosities since water molecules diffuse into the material, triggering the chemical degradation and resulting in formation of degradation products, which can be released from the material leading to mass loss. [33-35]

However, our results contradicted the results obtained by Okuda *et al.,* regarding silver uptake (nanoleakage) by the Single Bond adhesive which did not increase during the four time periods (one day, three, six and nine months). The bond strength gradually decreased over time due to the decrease in the physical properties of bonding resin occurring at a faster rate than degradation of the interface between the hybrid layer and dentin. [27] Also, Li *et al.,* revealed that, after three months storage, the leakage pattern revealed a similar pattern to that at 24 hours. While silver deposition was slightly more after six months and became greater after 12-months storage. This may be related to the slow absorption of water and/or the hydrolysis of the adhesive resins. [26]Furthermore, Yap *et al.,* found that, at all time intervals (24 hours, 1, 2, 3 and 4 weeks), no significant difference in gap widths was observed between all tested materials which was attributed to the hygroscopic expansion of the composites/bonding systems. [36]

Conclusions:

Under the test conditions, BOND 1 SF provided better sealing ability. Meanwhile, the tested home bleaching gel increased nanoleakage for both tested adhesives. Also, nanoleakage increased by aging.

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