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Long-Term Nanoleakage Depth and Pattern of Cervical Restorations Bonded With Different Adhesives

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Clinical Relevance

A mild acetone-based single-step self-etched adhesive system may reveal less nanoleakage in the short-term interval, but unfortunately, this was not sustained after long-term storage.

SUMMARY

Purpose: This study investigated the effect of water storage on nanoleakage depth and the pattern of cervical cavities bonded with different adhesives.

Methods: For nanoleakage depth evaluation, standardized cervical cavities (2 mm in diameter) were prepared on the buccal and lingual surfaces of 36 intact human premolars. Specimens were divided into three groups (n=12) according to the three adhesive systems used: an etch-and-rinse adhesive (SBMP, Adper Scotchbond Multi-Purpose, 3M ESPE) and two single-step self-etch adhesives; one was mild and acetone based (IB-iBond, Kulzer), while the other was strong water based (PL, Adper Prompt L-Pop, 3M ESPE). All cavities were restored using Filtek Z250 (3M ESPE) resin composite. For each adhesive, specimens (n=12 with 24 restored cavities) were subdivided into three subgroups (n=4 with eight cavities) according to the storage period before examination (24 hours, three or six months). Another duplicate of teeth was prepared in the same way for nanoleakage pattern evaluation. After storage, the specimens were placed in 50%W/V silver nitrate solution for 24 hours and immersed in a photo-developing solution for eight hours. Thereafter, the specimens were sectioned buccolingually, polished, and examined by scanning electron microscopy. For nanoleakage pattern, specimens were treated in the same way as for nanoleakage depth except that they were additionally immersed in 10% EDTA for five seconds after

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polishing. Silver penetration percentage was calculated to the total length of the tooth-restoration interface. Data were analyzed with two-way analysis of variance, Kruskal-Wallis, and post hoc tests.

Results: After 24 hours, the least amount of nanoleakage depth was recorded for IB, while the highest was recorded for PL. For stored specimens, there was no significant difference among the nanoleakage depths of all adhesives. The tested adhesives recorded different nanoleakage patterns; however, there was an increase in the intensity and continuity of silver deposition by time.

Conclusions: After 24 hours, the nanoleakage depth/pattern varied with the type of adhesive used; however, after water storage, all adhesives performed equally.

INTRODUCTION

The utmost goal of bonded restorations is to achieve a marginal and internal seal. Microleakage is defined as the leakage of fluids at the tooth-restoration interface through a gap.[1] Failure to seal the restoration margin can result in postplacement sensitivity, margin staining, and recurrent caries, which are the most common reasons associated with clinical failure of adhesive restorations.[2] Fluid leakage without gap formation can also occur. Nanoleakage is an internal leakage describing the nanometer-sized spaces around the collagen fibrils within the hybrid layer.[3] Authors have also reported that nanoleakage increases with long-term storage.[4] Although there is still no clear evidence of the negative effects of nanoleakage, the existence of such a pathway in gap-free cavity margins may have potential long-term consequences for adhesion quality.[5]

A number of new adhesive systems have been developed in an attempt to obtain a reliable adhesive-restoration interface over time.[6] Current adhesive systems interact with tooth substrate, using two different strategies. The total-etch technique implies removal of the smear layer. However, incomplete expansion of collagen may impair resin infiltration and compromise bonding with those adhesives.[7] The self-etch adhesive strategy is based on the simultaneous etching, priming, and bonding to the smear-covered dental tissues.[8] When using all of these self-etch adhesive systems, less discrepancy is expected between the depth of demineralization and depth of resin infiltration. Meanwhile, in an attempt to reduce the clinical application steps as well as the technique sensitivity, single-step self-etch adhesives have been introduced in the market.[9]

Single-step self-etch adhesives vary in their acidity by virtue of the composition and concentration of polymerizable acids and acidic resin monomers.[10,11] Strong self-etch adhesives were characterized by their better etching performance on enamel and compromised bonding to dentin. Mild self-etch adhesives rely on keeping the hydroxyapatite at the interface protecting the collagen and allowing for chemical interaction. The resultant twofold micro-mechanical and chemical bonding mechanisms of mild adhesives were more promising regarding the bond strength.[7] However, the nanoleakage performance of these different types of self-adhesive systems is not yet clear. Therefore, this study was conducted to compare the nanoleakage depth and pattern of bonded cervical cavities using strong or mild self-etch adhesives over time.

MATERIALS AND METHODS

The material brand names (manufacturer), description, and composition (lot number) are listed in Table 1. For the present study, freshly extracted human premolars free from caries or any cracks were collected. A hand scaler was used to remove the remnants of the periodontal tissues and calculus if present. The selected teeth were stored in a phosphate-buffered solution containing 0.8% sodium azide for a maximum period of one week until use.[12]

Specimen Grouping and Preparation

For nanoleakage depth evaluation, 36 teeth were divided into three main groups of 12 teeth each, according to the three adhesive systems used. For each adhesive system, the teeth were subdivided into three subgroups of four teeth each, according to the storage period before examination (24 hours, three or six months). In each tooth, two cavities were prepared (buccal and lingual), yielding eight cavities for each adhesive system tested at each storage period. Another duplicate of teeth was prepared in the same way for the nanoleakage pattern evaluation.

Standardized buccal and lingual cervical cavities were prepared. For each tooth, the cemento-enamel junction (CEJ) was marked with a pencil. The mesiodistal width was measured using a precise digital caliber (Mitutoyo, Digimatic Caliper, Mitutoyo Corp, Tokyo, Japan). The cavities were designed to coincide with the points of intersection between the drawn midlines on the buccal and the lingual

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Table 1: *Material Brand Names (Manufacturer), Description, Composition (Lot #), and Bonding Procedures*

Brand Name (Manufacturer)	Description	Composition (Lot #)	Bonding Procedure
Adper Scotchbond Multi-Purpose Adhesive System (3M ESPE, St Paul, MN, USA)	Three-step etch-and-rinse adhesive system	Etchant: 35% phosphoric acid (20031106)	Applied 15 seconds to enamel and dentin; rinsed thoroughly; gently air dried; dentin was left moist
		Primer: HEMA, polyalkenoic acid copolymer and water (20030408)	Applied; gently air dried for three seconds
		Adhesive: HEMA and Bis-GMA (20031106).	Applied and light cured for 20 second.
Adper Prompt L-Pop adhesive system (3M ESPE)	Two-component, single-step, self-etch adhesive system	Liquid 1 (red blister): methacrylated phosphoric esters Bis-GMA, initiators based on camphorquinone, stabilizers	Two components were mixed starting with pressing red reservoir toward yellow one; applied to enamel and dentin; rubbed for 15 seconds; gently air dried for five seconds and light cured for 20 seconds
		Liquid 2 (yellow blister): Water, HEMA, polyalkenoic acid, stabilizers (I93938)	
iBond adhesive system (Heraeus Kulzer, Hanau, Germany)	One-component single-step, self-etch adhesive system	4-methacryloxyethyltrimetallitic anhydridw (4-META), UDMA, glutardialdehyde, acetone, water, photoinitiator (010049)	Shaken three seconds before use; applied to enamel and dentin in three consecutive layers; massaged for 30 seconds; gently air dried for five seconds and light cured for 20 seconds

Abbreviations: 4-META, 4-methacryloxyethyltrimellitic anhydride; Bis-GMA, bis-phenol-A glycidyl-methacrylate; HEMA, 2-hydroxyethyl methacrylate; UDMA, urethane dimethacrylate.

surfaces and the marked CEJ. This enabled the drilling of cavities to be exactly aligned. To standardize the cavity depth and diameter at 2 mm ± 100 μm, the cavities were prepared using round stones (Komet, Brasseler, GmbH, Germany) in a successive order (ISO 012, 014, 016, 018). The cavity dimensions were ensured using the same digital caliber.

Each adhesive system was applied according to the manufacturer's instructions (Table 1). Resin composite restorative material (Filtek Z250, 3M ESPE), shade A3.5, was inserted in one increment using a plastic instrument (Dentsply, Ash, and Surrey, England). Caution was taken to minimize the excess material over the cavity margins. A polyester strip was applied, and the restorative material was light cured for 40 seconds using a light-curing unit of ≥500 mW/cm^2 intensity, which was checked with a radiometer (Demetron LED Radiometer, SDS, Kerr, Orange, CA, USA). After curing the excess composite, flash was removed using a lancet (Wuxi Xinda Medical Devices Co Ltd, Wuxi, Jiangsu, China). Then, finishing was done using rubber finishing points (Ivoclar Vivadent, Schaan, Liechtenstein) and a magnifying lens (Baush and Lomb Optics Co, Rochester, NY, USA) of 6× magnification to ensure that there was no composite flash at the cavity margins. The restored teeth were immersed in distilled water at 23°C ± 2°C[13] and left according to the intended storage periods (24 hours, three or six months). Water storage was changed weekly.[14] Teeth were then prepared for nanoleakage evaluation.

Nanoleakage Evaluation

After storage, customized circular pieces of adhesive tape were cut 3 mm in diameter using a 72-revolving-punch plier (General, Montreal, Canada). Each piece was placed over the restored cavity protecting the filling and 1 mm around the cavity margins. Specimens conducted for nanoleakage depth evaluation were then coated with a double layer of a fast-setting nail varnish, leaving 1 mm

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Table 2: *Means and Standard Deviations for Silver Penetration Percentage Specimens Bonded With Different Adhesive Systems Over Different Storage Periods*[a]

Variable	SBMP	PL	IB	*p* Value
24 hours	5.2 ± 1.2^{aA}	9.3 ± 1.6^{bA}	4.8 ± 2.8^{aA}	≤ 0.01
Three months	11.6 ± 2.6^{aB}	12.3 ± 6.7^{aA}	11.8 ± 3.3^{aB}	≥ 0.05
Six months	12.2 ± 1.6^{aB}	11.3 ± 1.9^{aA}	9.8 ± 3.7^{aB}	≥ 0.05
p value	≤ 0.001	≥ 0.05	<0.05	

[a] *Rows with the same lowercase superscript letters are not statistically different from each other. Columns with the same uppercase superscript letters are not statistically different from each other.*

from the bonded interface exposed. The teeth were immersed in 50% silver nitrate solution for 24 hours in total darkness, rinsed thoroughly, and immersed in a photo-developing solution for eight hours under fluorescent light. Then, the teeth were rinsed using tap water for 60 seconds as described by Tay and others.[15] After that, the teeth were sectioned through the centers of the restorations. The cut surfaces were finished and polished with fine silicon carbide abrasive papers (Buehler, Lake Bluff, IL, USA) in an ascending order (from 600 grit and up to 4000 grit) and examined using a scanning electron microscope (SEM; Philips XL30-5600MD, Eindhoven, the Netherlands) in back-scattered mode. The teeth intended for nanoleakage pattern examination were treated in the same way as those for nanoleakage depth evaluation except that they were additionally immersed in 10% EDTA for five seconds after polishing.[13]

The specimens were mounted on aluminum studs, gold sputter coated, and examined in the back-scattered electron mode at an acceleration voltage of 25 kV. Evaluation of nanoleakage depth was done using SEM. The depth of silver penetration was measured as well as the total length of the bonded interface. The percentage of the penetration depth to the total length of the bonded interface was calculated and recorded. The teeth intended for nanoleakage pattern evaluation were examined using SEM at 500× or 800× magnification.

Statistical Analysis

Data were statistically described in terms of mean ± standard deviation. Two-way analysis of variance was used to test the complex interaction between adhesive system and storage time. This was followed by a Kruskal-Wallis test for multiple comparisons and Convene Inman test for pairwise comparisons as a post hoc test. *p* values less than 0.05 were considered statistically significant. All statistical calculations were done using the computer programs Microsoft Excel 2007 (Microsoft Corporation, Redmond, WA, USA) and Stats Direct statistical software version 2.7.2 for MS Windows (Stats Direct Ltd, Cheshire, UK).

RESULTS

The means and standard deviations and statistical significance of the percentage of silver penetration for the three adhesive systems at the three storage periods are presented in Table 2.

Regarding the nanoleakage depth, at 24 hours, Adper Prompt L-Pop recorded a statistically significant higher percentage of silver penetration than the other materials ($p \leq 0.01$). After storage for three and six months, specimens showed a significant increase in percentage values of silver penetration for both Adper Scotchbond Multi-Purpose ($p \leq 0.001$) and iBond ($p < 0.05$), respectively. For Adper Prompt L-Pop, no significant difference ($p \geq 0.05$) was found between the three storage periods. Also, there was no statistically significant difference ($p \geq 0.05$) in the percentage of silver penetration values among the three adhesives used at the three- and six-month storage periods.

The Adper Scotchbond Multi-Purpose nanoleakage pattern revealed silver patches at the base of the hybrid layer after 24 hours (Figure 1). After storage, silver patches increased in number and size. They were found anywhere within the thickness of the hybrid layer. Silver was never found in the adhesive layer for all the specimens (Figures 2 and 3). For Adper Prompt L-Pop, a thin hybrid layer was formed. Reticular silver deposits were observed in

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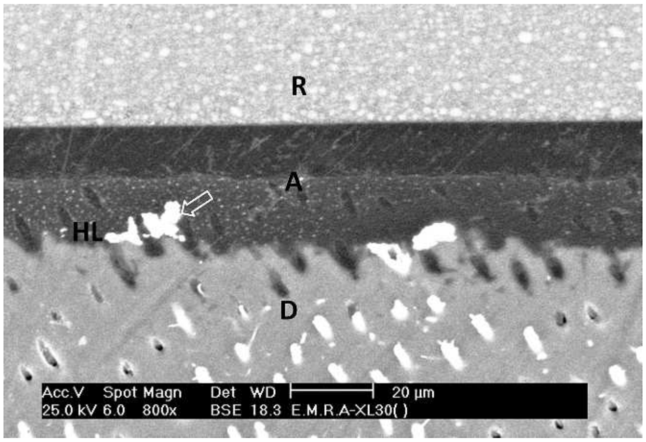

Figure 1. *Scanning electron microscope micrograph of Adper Scotchbond Multi-purpose adhesive dentin interface after 24-hour storage.*

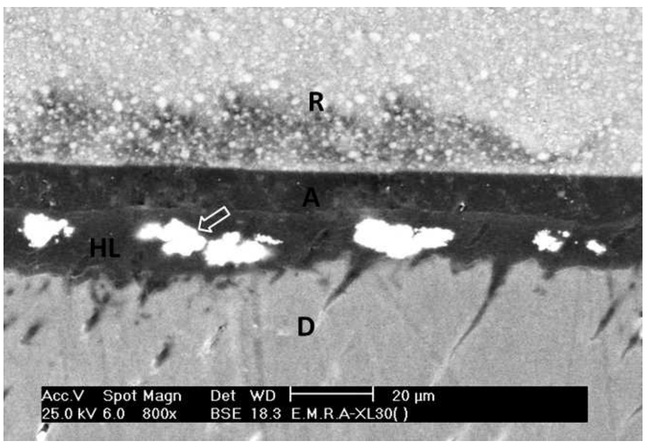

Figure 2. *Scanning electron microscope micrograph of Adper Scotchbond Multi-purpose adhesive dentin interface after three-month storage.*

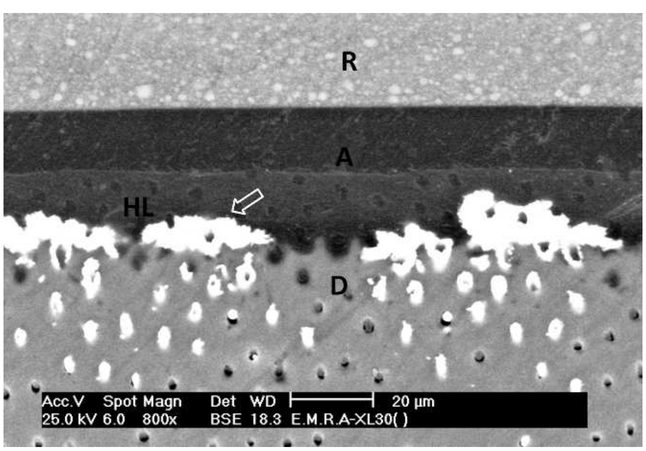

Figure 3. *Scanning electron microscope micrograph of Adper Scotchbond Multi-purpose adhesive dentin interface after six-month storage.*

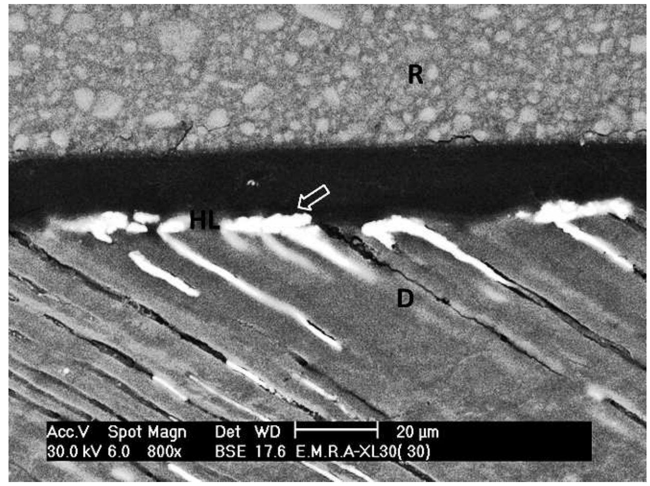

Figure 4. *Scanning electron microscope micrograph of Adper Prompt-L-Pop adhesive dentin interface after 24-hour storage.*

the 24-hour stored specimens (Figure 4). After 3-month storage, they became thicker and wider in spread (Figure 5). Upon storage for 6 months, reticular silver deposits continued to grow in width and height, and water trees became clearly observed (Figure 6). For iBond, the hybrid layer formed was very thin. Silver-stained bands (arrows) were found in the hybrid layer. No water treeing was observed in the 24-hour stored specimens (Figure 7). After aging, silver-stained bands became thicker and more continuous. Water trees (arrowheads) became more manifested within the adhesive layer (Figure 8) and increased in height and number after longer storage periods (Figure 9).

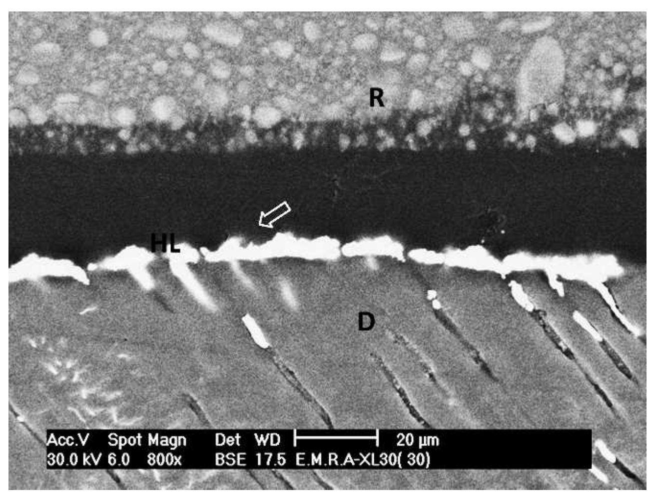

Figure 5: *Scanning electron microscope micrograph of Adper Prompt-L-Pop adhesive dentin interface after three-month storage.*

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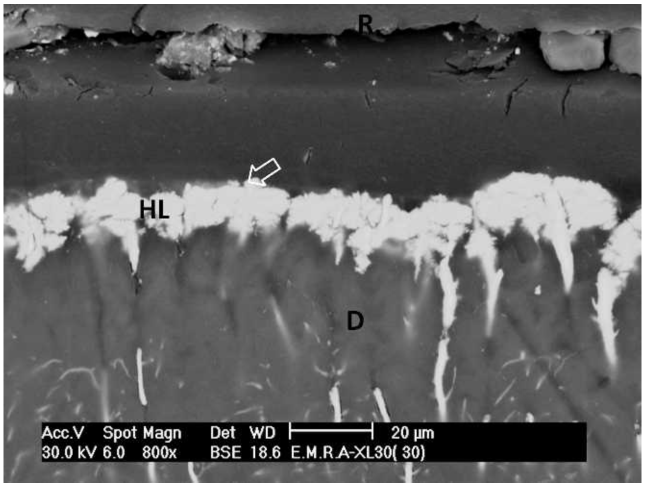

Figure 6. *Scanning electron microscope micrograph of Adper Prompt-L-Pop adhesive dentin interface after six-month storage.*

DISCUSSION

The use of bonded restorations is taking a growing role in restorative dentistry. The introduction of the acid etch technique was found to be enough to obtain acceptable sealing with enamel. However, obtaining sealed restorations at the dentin side without leakage was a more complicated target. Nanoleakage, which is a valuable criterion in the evaluation of adhesive performance, should be fully determined qualitatively as well as quantitatively.[16] In the current study, nanoleakage evaluation was carried out with high-magnification SEM by means of a back-scattered electron mode, which was reported to be better compared with the secondary electron images.[3] The present study was conducted on restored cervical cavities to evaluate nanoleakage of different adhesive strategies over different storage

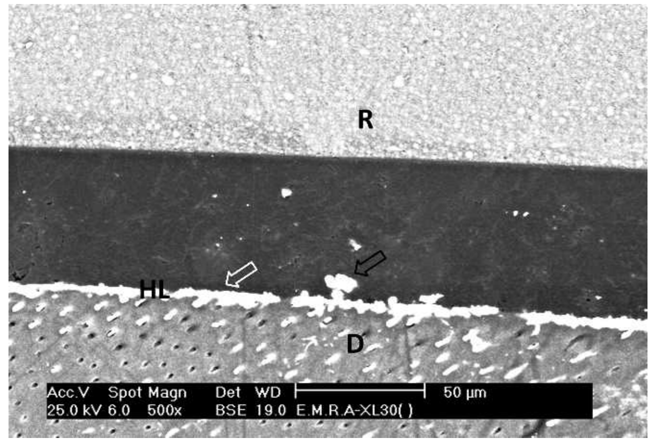

Figure 8. *Scanning electron microscope micrograph of iBond adhesive dentin interface after three-month storage.*

periods. The configurations of this cavity proved to be extremely challenging because of the high C-factor present. Most nanoleakage studies have used a flat dentin surface for bonding and nanoleakage evaluation.[4,12,13,15,17] However, cervical cavities represented a clinically relevant case.

For nanoleakage depth evaluation, the percentage of silver penetration to total length of bonded interface was calculated. This was in accordance with Li and others[18] and Fernando de Goes and Montes[19] and in opposition to other authors[20] who graded nanoleakage with scoring. The use of a scoring system, such as 0 = 0%, 1 = 1% to 25%, 2 = 25% to 50%, 3 = 50% to 75%, and 4 = >75%, presented in previous studies, was not preferred in the current study. This was because each score other than zero represented a wide range, making it unsuitable for differentiating between the tested adhesive systems as all would meet a score 1.

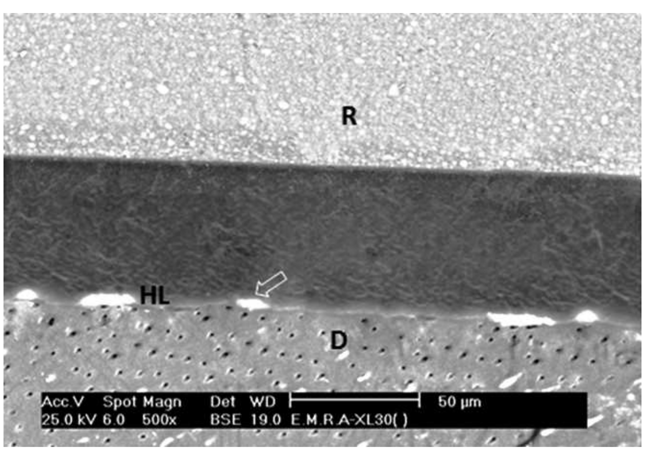

Figure 7. *Scanning electron microscope micrograph of iBond adhesive dentin interface after 24-hour storage.*

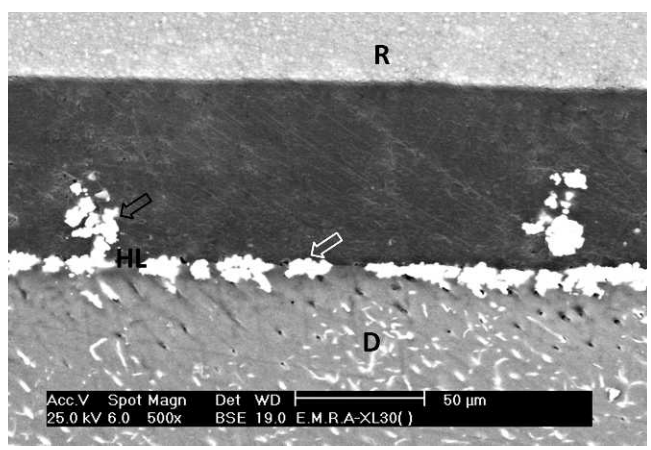

Figure 9. *Scanning electron microscope micrograph of iBond adhesive dentin interface after six-month storage.*

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The observation of the photomicrographs resulting from the back-scattered electron mode revealed a difference in nanoleakage among different adhesive systems. It is generally accepted that heavy silver uptake along the interfacial layer and in the adhesive layer can be due to imperfect resin infiltration, retained water or other solvent, poor polymerization, or phase separation.[17] Regarding the etch-and-rinse adhesive technique, De Munck and others[21] stated that none of the contemporary all-in-one adhesives can compete with the more traditional multistep adhesives. However, the reported nanoleakage could be attributed to many factors. It was speculated that this represents the presence of very thin shrunken collagen fibers, which may accumulate on the dentin surface following etching.[22] This thin layer (0.2-0.3 μm) might interfere with adhesive resin infiltration, and silver ions may precipitate within this collagen smear layer. In addition, more evaporative water flux is expected with the strong air blowing recommended by the manufacturer of the used adhesives in order to evaporate the solvent and remove the excessive interfacial water.

Although the self-etch adhesive strategy is highly promising as a user-friendly and less technique-sensitive adhesive,[23] simplification and saving time may be at the expense of compromising the quality of resin-dentin bonds.[24] Reasons for reported nanoleakage are numerous. Among them is that they are too hydrophilic and act, even after polymerization, as semipermeable membranes.[25] High solvent concentration makes it impossible to obtain an adhesive resin layer of adequate thickness and free from residual solvent.[21] Phase separation and blistering that may occur during solvent evaporation due to change in the monomer-water ratio can also be a cause.[26] Additional explanations were added by Toledano and others.[27] One of them is the combination of acidic hydrophilic and hydrophobic monomers into a single step that compromises the polymerization of the adhesives.

Another investigation finding in the present study regarding self-etch adhesives was that the iBond self-etch adhesive showed significantly low nanoleakage penetration depth values after 24 hours compared with Adper Prompt L-Pop. The difference in silver deposition may be regarded as the difference in the acidity of adhesives tested and the chemical nature, which in turn affect the degree of water sorption and the bonding efficacy. The low pH ($pH = 0.40$ of Adper Prompt L-Pop[28]) denotes a high concentration of acidic uncured resin monomers. Another reason may be the low viscosity of Adper Prompt L-Pop that results in its spread so thin as to lead to formation of dry spots.[29] iBond ($pH = 2$) is an acetone-water–based adhesive,[28] whereas Adper Prompt L-Pop is water based. Water is comparatively easy to remove with the use of acetone-based adhesives because acetone increases the vapor pressure of water.[30] Also, iBond is HEMA free in contrast to Adper Prompt L-Pop. HEMA lowers the vapor pressure of water when added to a water mixture, making it more difficult to remove water from the adhesive and increasing water retention within the adhesive layer.[23]

Concerning the storage factor, specimens stored for three or six months had a significantly higher nanoleakage depth in comparison to 24-hour tested specimens for iBond and Adper Scotchbond Multi-Purpose adhesive systems. For Adper Prompt L-Pop, no significant difference was found during different storage periods. Also, the present study revealed an insignificant difference in the silver penetration percentage between three- and six-month storage regardless of the adhesive type. Such a trend has also been reported by Okuda and others.[31] They found that there was no statistically significant difference in silver penetration for the tested adhesive systems among three-, six-, and nine-month periods. These results denote that the dentin-resin interface deteriorates over time.

Bond degradation is divided into two phases: hydrolytic degradation of the collagen matrix and hydrolytic degradation of the bonding resin within the hybrid layer. Water has been claimed as one of the major causes of the collagen and resin degradation that occurs overtime. Hydrolysis is a chemical process that breaks covalent bonds between the polymers by addition of water to ester bonds, resulting in loss of resin mass.[4] Water sorption is enhanced by the presence of hydrophilic and ionic resin monomers, which in turn facilitates ion movement within a polymerized resin matrix.[32] The combined degradation of resin and collagen may increase the water content of the bonded interface, leading to a further detrimental effect on the longevity of the bond. A further contributing factor to the degradation of the hybrid layer is endogenous matrix metalloproteinases (MMP) such as MMP-2, -8, -9, and -20.[11,33,34] MMPs are slowly released from the denuded demineralized dentin matrix even in the absence of bacteria, in a way that is similar to caries progression.[33]

The nanoleakage pattern was reported to be dependent on the adhesive tested.[22] In the present

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study, silver patches were seen in the hybrid layer formed by Adper Scotchbond Multi-Purpose, with the absence of silver staining in the adhesive layer. These results were in accordance with Hashimoto and others.[13] The reticular pattern of silver penetration that was observed in the hybrid layer of Adper Prompt L-Pop and the water trees in the adhesive layer were in agreement with Reis and others.[35] Silver-stained bands were found in the hybrid layer formed by iBond. Water trees were also seen in the adhesive layer of iBond. Such findings may denote that the C-factor has no effect on the nanoleakage pattern. Based on this, in the clinical situation, it is preferred that adhesive systems are hydrophilic during application and then become hydrophobic after application and completely seal the restoration margins. The dream of each researcher and clinician to have an adhesive system suitable for any case and revealing a marginal and internal seal is yet to be fulfilled.

CONCLUSIONS

Under the conditions of this study, the following can be concluded:

1. Nanoleakage is dependent not only on the application technique but also on the adhesive chemical nature.
2. Storage in distilled water increased nanoleakage depth, continuity, and intensity.

Conflict of Interest Declaration

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

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