

The nanoleakage patterns of experimental hydrophobic adhesives after load cycling

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ABSTRACT

The purpose of this study was: (1) to compare nanoleakage patterns of a conventional 3-step etch and rinse adhesive system and two experimental hydrophobic adhesive systems and (2) to investigate the change of the nanoleakage patterns after load cycling. Two kinds of hydrophobic experimental adhesives, ethanol containing adhesive (EA) and methanol containing adhesive (MA), were prepared. Thirty extracted human molars were embedded in resin blocks and occlusal thirds of the crowns were removed. The polished dentin surfaces were etched with a 35 % phosphoric acid etching gel and rinsed with water. Scotchbond Multi-Purpose (MP), EA and MA were used for bonding procedure. Z-250 composite resin was built-up on the adhesive-treated surfaces. Five teeth of each dentin adhesive group were subjected to mechanical load cycling. The teeth were sectioned into 2 mm thick slabs and then stained with 50 % ammoniacal silver nitrate. Ten specimens for each group were examined under scanning electron microscope in backscattering electron mode. All photographs were analyzed using image analysis software. Three regions of each specimen were used for evaluation of the silver uptake within the hybrid layer. The area of silver deposition was calculated and expressed in gray value. Data were statistically analyzed by two-way ANOVA and post-hoc testing of multiple comparisons was done with the Scheffe's test. Silver particles were observed in all the groups. However, silver particles were more sparsely distributed in the EA group and the MA group than in the MP group ($p < .0001$). There were no changes in nanoleakage patterns after load cycling. [J Kor Acad Cons Dent 33(1):9-19, 2008]

Key words : Gray value, hydrophobic adhesive, load cycling, nanoleakage

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I . INTRODUCTION

Bonding to dentin has been one of the most important and challenging issues in restorative

dentistry since the introduction of adhesive technology nearly 50 years ago¹⁾. The process of bonding to dentin generally begins with acid etching, which is necessary to remove the smear layer and to create a 1 - 5 μm -deep demineralized zone in the dentin surface. In the next step, hydrophilic primers are applied, which diffuse across the demineralized layer to displace water and stabilize the collagen network. Finally, the adhesive resins are applied to the primed dentin and polymerized. The hybrid layer or resin-dentin interdif-

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fusion zone is formed based upon this micro-mechanical interlocking, which is considered the most important mechanism for dentin bonding²⁾.

Dentin can be regarded as a biological composite of a collagen matrix which is highly filled with nanometer-sized apatite crystal. After extracting the mineral from the collagen fibrils, the voids can be filled with a resin, thus forming a new composite made up of resin matrix filled with a fibrous collagen³⁾.

In order to obtain consistent hybrid layers, the three-dimensional structure of a collagen network should be exposed by acid etching and the interfibrillar space of the collagen network should be maintained by being floated in water during infiltration of resin monomers. Collapse of collagen network reduces the porosity of the demineralized dentin, inhibits resin penetration through the demineralized layer⁴⁾ and forms a barrier between the demineralized layer and the underlying intact or unreacted dentin surface. Because optimal interfacial bonding can be achieved by resin diffusion through the conditioned dentin, it is essential to maintain collagen network without collapse⁵⁾.

The lack of complete diffusion throughout the demineralized dentin with adhesive systems that inhibit collagen collapse through "wet" bonding can be attributed to a variety of factors, including dissimilar solvents and differences in the hydrophobicity of the adhesive. For example, under moist bonding conditions, the channels between demineralized dentin collagen fibrils are filled with water. The only mechanism available for monomer infiltration is diffusion of monomers into whatever solvent is in the spaces of the substrate and along the collagen fibers³⁾.

This dentin bond, if still intact after resin composite placement and polymerization, can degrade over time, leading to clinical restorative failures. Biodegradation of the collagen matrix and/or synthetic resin components of this hybrid layer is possibly due to incomplete penetration/infiltration of resin into the dentin substrate, heterogeneous distribution of monomers through the interdiffusion zone, suboptimal polymerization and hydrolysis⁶⁾.

In order to improve the durability of dentin adhesives, it is necessary that complete removal of water from the exposed collagen network without deteriorating the integrity of its three-dimensional structure and substitution of hydrophilic monomers in the adhesives with more hydrophobic monomers which can resist hydrolysis. Most primers in currently available systems usually contain hydrophilic monomers, such as 2-hydroxyethyl methacrylate (HEMA), as surface-active agents to enhance the wettability of the hydrophobic adhesive resins. HEMA can polymerize in the presence of water to form 'microporous' hydrogel (polyHEMA) with pore sizes ranging from 10 to 100 nm⁷⁾. The loss of interfibrillar resin may have been caused by slow hydrolysis of polyHEMA⁸⁾. Like HEMA, alcohols at higher concentration also significantly stabilized collagen⁹⁾ and high vapor pressure of these solvents have been used to compete with and displace water and to facilitate the permeation of monomers¹⁰⁾.

In order to increase the durability of dentin-adhesive interfaces, by priming the acid-etched dentin surfaces with organic solvents, we tried to remove water from the exposed collagen network without collapse. We also tried to penetrate the experimental adhesives containing relatively hydrophobic monomers into the collagen network floating in the solvent.

The hypothesis tested was that priming the collagen network with organic solvents might displace water from it without collapse and thereby represent less nanoleakage.

The purpose of this study was (1) to compare nanoleakage patterns of a conventional 3-step etch and rinse adhesive system and two experimental hydrophobic adhesive systems and (2) to investigate the change of the nanoleakage patterns after load cycling.

II. MATERIAL AND METHODS

Preparing experimental adhesives

Scotchbond Multi-Purpose (3M ESPE, St. Paul, MN, USA) was used as control. Two experimental adhesives were prepared by dissolving a mixture

Table 1. Materials and compositions of adhesive systems

Adhesive system	Primer	Adhesive Resin
Scotchbond Multi-Purpose	HEMA, light-cured polymer, water	Bis-GMA, HEMA, CQ, 4E
Ethanol containing adhesive	99.9% ethanol	Ethanol, Bis-GMA, TEGDMA, CQ, 4E
Methanol containing adhesive	99.9% methanol	Methanol, Bis-GMA, TEGDMA, CQ, 4E

(1 : 1) of two hydrophobic monomers, Bis-GMA (Bisphenol-A-glycidyl dimethacrylate, Shin-Nakamura chemical Co., Inc.) and TEGDMA (Triethyleneglycol dimethacrylate, Shin-Nakamura chemical Co., Inc.), into an equal amount of an organic solvent (50 wt%), one of ethanol and methanol. Camphoroquinone (CQ) and Ethyl 4-dimethylaminobenzoate (4E) were each added to the mixture by 1.0 wt% of resin monomers (Table 1).

Bonding Procedures

Thirty freshly extracted human molars stored in 0.5 % chloramine T solution were used. Occlusal enamel and coronal dentin were removed using a low-speed saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) under running water. The exposed dentin surfaces were wet ground with 500 grit silicone carbide paper (Rotapol-V, Struers Ltd, Glasgow, UK) for 60 seconds. The three dentin adhesive systems were used in this study (Table 1). Scotchbond Multi-Purpose (MP) and two experimental adhesive systems, ethanol containing adhesive (EA) and methanol containing adhesive (MA), were used. The polished dentin surface was etched with a 35 % phosphoric acid etching gel (3M ESPE, St. Paul, MN, USA) for 15 seconds and rinsed with distilled water for 15 seconds. Ten teeth were used for each adhesive. In the MP group, dentin surfaces were treated following the manufacturer's instruction. In the EA and MA groups, immediately after rinsing, the teeth were primed with ethanol or methanol for 15 seconds and then the experimental adhesives containing the same solvent as the priming solvent were applied on the solvent-primed dentin with two successive coats and agitated for 10 seconds. The remaining solvent was dried with gen-

tle air blow. The adhesive was cured for 20 seconds using a dental light-curing unit (Hilux™ Ultra+, Benlioglu Dental Inc., Ankara, Turkey, power density: > 500 mW/cm²). Z-250 hybrid composite resin (A2 shade, 3M ESPE, St. Paul, MN, USA) was built-up in two increments on the adhesive-treated surfaces to 4.0 mm thickness (Table 2).

Load Cycling

Five teeth of each dentin adhesive group were subjected to mechanical load cycling. The cyclic mechanical loading device used was a hydraulic dynamic fatigue testing machine (858 Bionix II, MTS, USA). The tooth was mounted in acrylic resin block and 100N was loaded on the middle of each tooth by a steel tip attached to the machine for 1,000,000 cycles in water at room temperature.

Nanoleakage evaluation

The teeth were sectioned occluso-gingivally perpendicular to the bonded interface into approximately 2.0 mm thick slabs using a diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) under water cooling. Two slabs were obtained from each tooth. And then the slabs were coated twice with nail varnish except within 1 mm of the bonded interfaces. The slabs were placed in 50 % (w/v) silver nitrate solution in total darkness for 24 hours, rinsed in running water for 5 minutes, and immersed into photo-developing solution under fluorescent light for 8 hours in order to reduce the silver ions to metallic silver. After removal from the developing solution, the teeth were placed in running water for 5 minutes¹¹⁾. Specimens were polished with increasingly fine diamond pastes (6, 3 and 1 µm particles, Buehler

Table 2. Bonding Procedure

Scotchbond Multi-Purpose	Etching : apply etchant for 15 sec, rinse for 10 sec, blot dry
	Priming : apply primer for 15 sec, gentle air dry
	Bonding : apply adhesive, gentle air thinning, light-cure for 20 sec
Ethanol containing adhesive	Etching : apply etchant for 15 sec, rinse for 10 sec, blot dry
	Priming : apply ethanol for 15 sec
	Bonding : apply adhesive (two coats), gentle air thinning, light-cure for 20 sec
Methanol containing adhesive	Etching : apply etchant for 15 sec, rinse for 10 sec, blot dry
	Priming : apply methanol for 15 sec
	Bonding : apply adhesive (two coats), gentle air thinning, light-cure for 20 sec

Ltd., Lake Bluf, IL, USA). The specimens were cleaned ultra-sonically, air dried, mounted on aluminum stubs, placed in a desiccator for 24 hours, and coated with thin gold. Ten specimens for each group were examined under the scanning electron microscope (SEM, S-4700 Hitachi, Japan) in backscattering electron mode.

The image analysis method was modified from a protocol previously described by Tay et al¹²⁾. All photographs were analyzed using image analysis software (Scion Image Beta 4.03, Scion Corp., Frederick, MD, USA)¹³⁾. The amount of silver nitrate within the hybrid layer was measured with gray value in three regions ($3.0\ \mu\text{m} \times 3.0\ \mu\text{m}$) of the bonded interface (left, center and right of each specimen). In SEM image, silver nitrate was observed as white particle and the silver nitrate uptake was expressed as average gray value ranging from 0 (black) to 255 (white). Data were statistically analyzed by two-way ANOVA and post-hoc testing of multiple comparisons was done with the Scheffe's test.

III. RESULTS

All specimens in the six groups showed nanoleakage at the adhesive/ dentin interface. Typical leakage patterns at the resin-dentin interfaces for each dentin adhesive system are illustrated in Figures 1 - 7.

The Scotchbond Multi-Purpose system produced a very distinct hybrid layer which was approximately $3\ \mu\text{m}$ thick. Distinct resin tags were also

observed as shown in Figures 2 and 3. Silver particles were deposited along the lower half of the hybrid layer that were different, however, from those of the experimental adhesive systems. The leakage pattern revealed a thin but very dense deposition of silver at the bottom of the hybrid layer. There was also distinct deposition of silver between the resin tag and tubule walls. The average gray scale was 91.78 in the non-load cycling group and 110.11 in the load cycling group (Table 3). The Scotchbond Multi-Purpose system produced an approximately $300\ \mu\text{m}$ thick adhesive layer (Figure 1a).

The leakage patterns for the ethanol containing adhesive were shown in Figures 4 and 5. The hybrid layer was approximately $3\ \mu\text{m}$ thick. There were diffuse silver deposits within the hybrid layer, and dentinal tubules rarely took up silver. The average gray scale was 20.70 in the non-load cycling group and 24.20 in the load cycling group (Table 3). This system produced an approximately $50\ \mu\text{m}$ thick adhesive layer (Figure 1b).

The leakage patterns for the methanol containing adhesive are shown in Figures 6 and 7. The hybrid layer was approximately $3\ \mu\text{m}$ thick. Silver particles were evenly deposited within the hybrid layer, but of relatively low density. The tubule walls rarely took up silver particles. The average gray scale was 32.88 in the non-load cycling group and 32.27 in the load cycling group (Table 3). This system produced an approximately $30\ \mu\text{m}$ thick adhesive layer (Figure 1c).

The Scotchbond Multi-Purpose groups (non-load

Table 3. Mean gray values obtained on hybrid layer with or without load cycling with different adhesive systems

	Scotchbond Multi-Purpose	Ethanol containing adhesive	Methanol containing adhesive	p-value
Non-load cycling	91.78 ± 40.68 (30) ^a	20.70 ± 22.89 (27) ^b	32.88 ± 25.94 (30) ^b	0.0001
Load cycling	110.11 ± 33.17 (27) ^a	24.20 ± 24.52 (30) ^b	52.50 ± 45.43 (30) ^b	0.0001
p-value	0.390	1.000	1.000	

* The numbers in parentheses are the sample numbers.

* Groups having different letters (a,b = row) are significantly different ($p < .0001$)

* Non-load cycled groups and load cycled groups are not significantly different

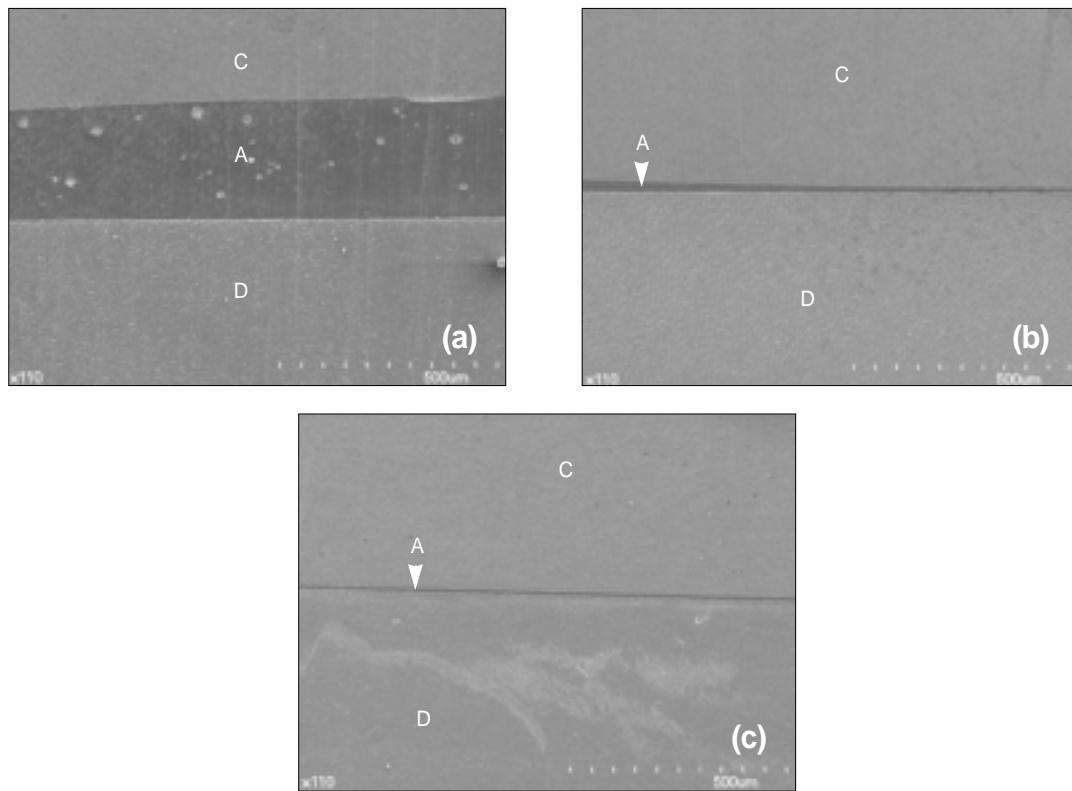


Figure 1. Backscattering SEM images at low magnification (110 ×). (a) Specimens bonded with Scotchbond Multi-Purpose demonstrate relatively thick adhesive layer. (b) Specimens bonded with ethanol containing adhesive demonstrate thin adhesive layer. (c) Specimens bonded with methanol containing adhesive demonstrate thin adhesive layer. A, Adhesive; C, Composite; D, Dentin.

cycling and load cycling) had significantly higher silver content than other groups ($p < 0.0001$) (Table 3). SEM images showed that samples sub-

jected to load cycling had leakage patterns similar to non-load cycled samples for all dentin bonding systems.

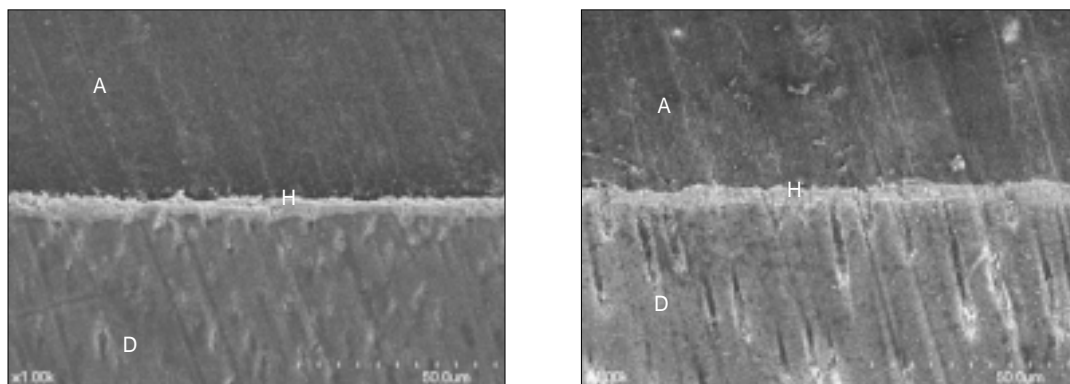


Figure 2. Backscattering SEM images (1000 \times) of specimens bonded with Scotchbond Multi-Purpose without load cycling. Silver particles were more densely deposited at the bottom of the hybrid layer even without load cycling.

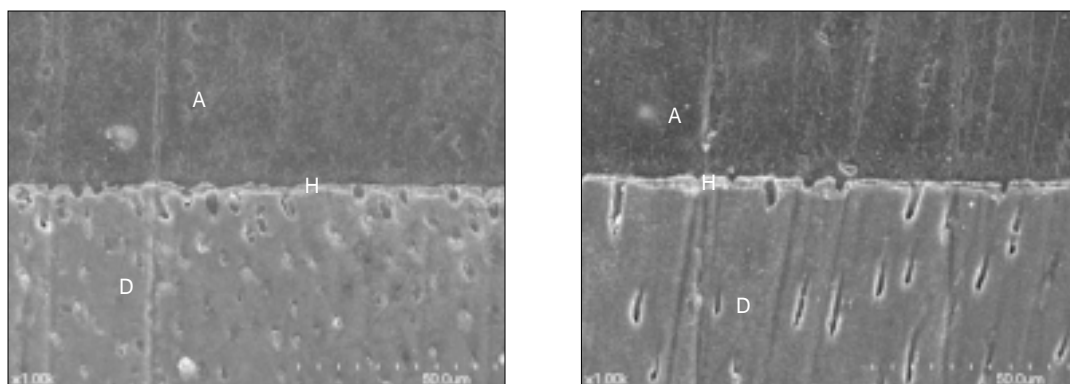


Figure 3. Backscattering SEM images (1000 \times) of specimens bonded with Scotchbond Multi-Purpose after load cycling. Dense silver deposition was noted at the bottom of the hybrid layer and silver particles were also densely deposited around the resin tags. Note that there were little differences in nanoleakage patterns between Figure 2 and Figure 3.

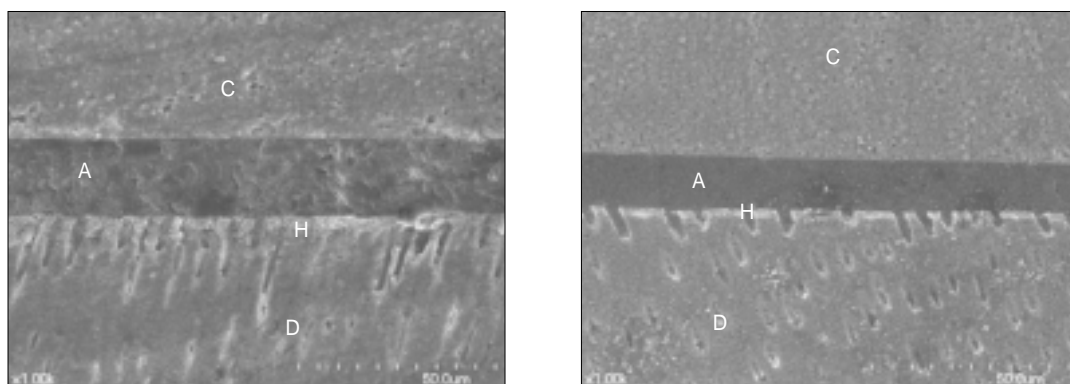


Figure 4. Backscattering SEM images (1000 \times) of specimens bonded with ethanol containing adhesive without load cycling. Silver particles were evenly deposited within hybrid layer. Relatively low density of silver particles were observed around the resin tags.

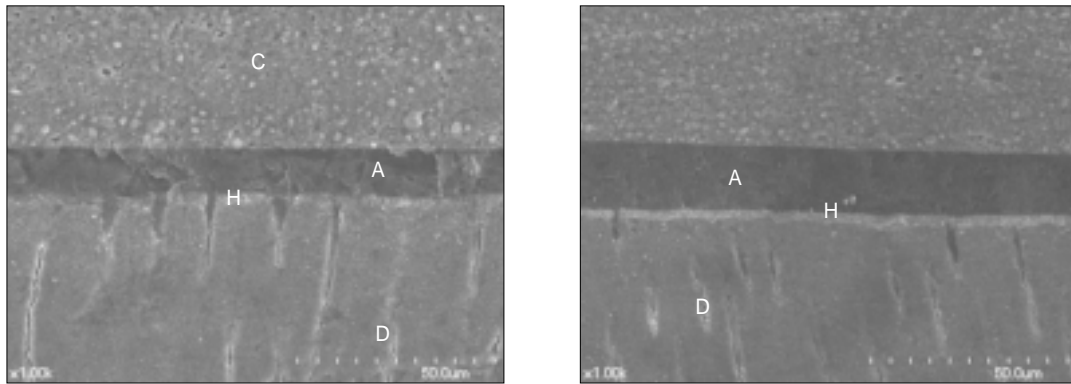


Figure 5. Backscattering SEM images (1000 ×) of specimens bonded with ethanol containing adhesive after load cycling. Note that there were no differences in nanoleakage patterns between Figure 4 and Figure 5.

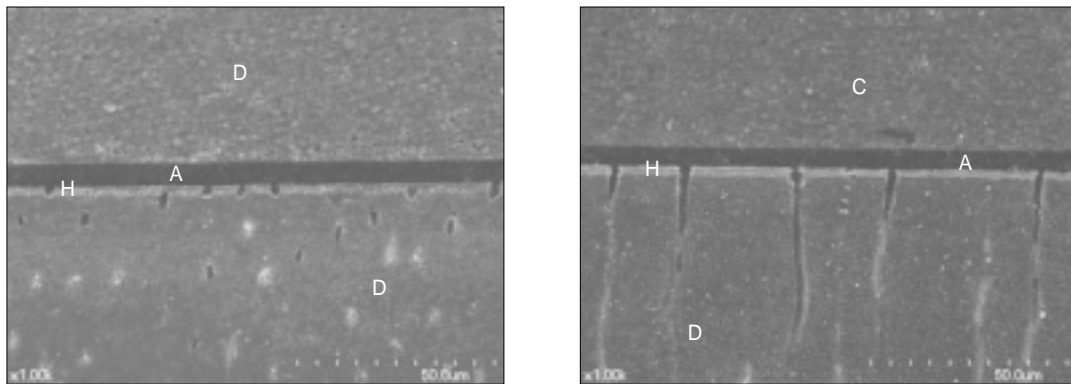


Figure 6. Backscattering SEM images (1000 ×) of specimens bonded with methanol containing adhesive without load cycling. Loose silver deposition within the hybrid layer was observed. Sparse silver deposition was observed around the resin tags.

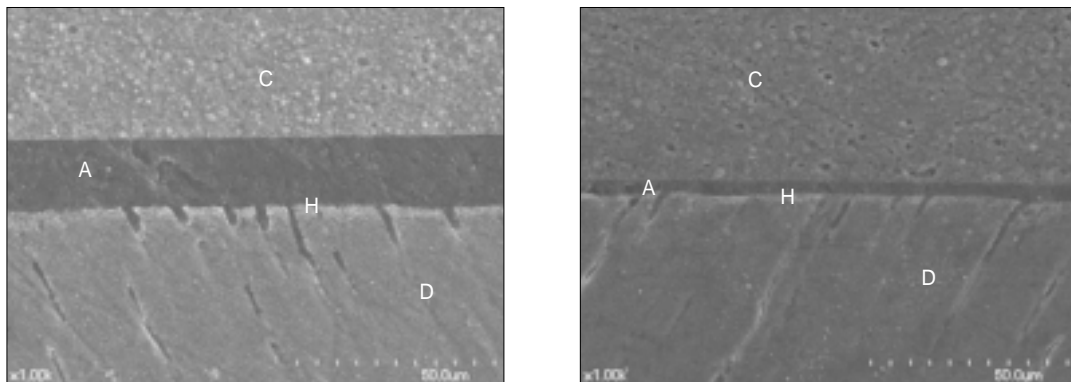


Figure 7. Backscattering SEM images (1000 ×) of specimens bonded with methanol containing adhesive after load cycling. There were no differences in nanoleakage patterns between Figure 6 and Figure 7.

IV. DISCUSSION

The durability of the bond between adhesive resins and dentin is of critical importance for the longevity of bonded restorations¹⁴⁾. This study was designed to evaluate whether new experimental adhesive systems, which contain less hydrophilic domains, might resist hydrolytic de-gradation. Sano et al.^{15,16)} hypothesized that nanoleakage revealed the location of defects at the resin-dentin interface, and could be the pathway for degradation of resin/dentin bonds over time.

All adhesives presented a certain degree of nanoleakage with differences among densities and patterns of silver deposition, depending on their composition. This may be a consequence of incomplete penetration of the adhesive resin into the demineralized dentin, leaving the collagen not enveloped by resin, or this may result from incomplete polymerization of resin monomer¹⁶⁾. The nanoleakage pathway may be located within the hybrid layer, within demineralized dentin or within the hydrophilic domain which exists in adhesive resin¹⁷⁾. Although nanoleakage was shown to occur throughout the hybrid layer and/or adhesive resin, the clinical significance of nanoleakage was unclear. The spaces are too small to allow bacterial penetration, but they were large enough for enzymes or bacterial product to enter.

The original interpretation of nanoleakage was that silver occupied nanometer-sized spaces around naked collagen fibrils, where resin failed to infiltrate, or where residual water had not been displaced by adhesive resin^{18,19)}. But Tay and Pashley¹²⁾ demonstrated that water could pass from dentin, around resin tags, to form water-filled channels that project from the hybrid layer into the overlying adhesive. When these water-filled channels are stained with silver, those often look like microscopic trees. They called them "water trees" and suggested that those might act as potential sites for hydrolytic degradation of resin/dentin bonds. Thus far, all marketed products permitted some amount of nanoleakage and water-tree formation.

In this study, there were much less silver depositions in the experimental adhesive groups. Ideally, HEMA, which is a primary component in many commercial dentin adhesive systems, conditions the collagen to remain expanded during adhesive infiltration^{20,21)}. However, results from a recent study indicate that HEMA can dramatically reduce the evaporation of water²²⁾. The addition of HEMA reduces the mole fraction of water and therefore reduces the partial pressure of water according to Dalton's law of partial pressures. As the partial pressure of water drops, it becomes more and more difficult to remove residual water from the demineralized dentin. Hydrophobic monomers, such as Bis-GMA, would resist diffusing into these sites where there is residual water⁵⁾. Because of high vapor pressure of alcohol solvents that were used as priming material in the experimental groups, the solvents might have competed with water and effectively replaced it. Therefore, the collagen network was expected to shrink much less and not to collapse, and then solvents might carry the monomers effectively into the spaces and facilitate the development of a hybrid layer¹⁰⁾. Furthermore, the alcohols and monomers used in the experimental adhesives had hydroxyl groups. The hydrogen-bonding between the hydroxyl groups might facilitate their penetration.

In the experimental adhesive groups, the adhesive layer was thinner than that of the Scotchbond Multi-Purpose group. In substance, the viscosity of both experimental adhesives was relatively low and it was very difficult to apply adhesive evenly. Therefore, this must be improved for clinical use.

Teeth are continually subjected to stresses during chewing, swallowing, and parafunctional habits. Vertical loading introduced by a food bolus between opposing teeth can be evenly distributed over the entire occlusal surface. Mechanical load cycling has been studied due to its potential capability of simulating mastication^{23,24)}. Anderson²⁵⁾ recorded axial loads from 70 to 150 N during chewing and swallowing. A load of 100 N was used in this study, as it was considered to be

within the normal functional range.

In this study, the load cycling did not influence the leakage pattern of each dentin adhesive system. For micro-leakage evaluation, discrepant results related to the effect of load cycling have been reported²⁶⁻²⁹. Other studies, however, found no effect of load cycling on nanoleakage, which is in accordance with the finding of this study^{23,30}. After hybridization of the adhesive and demineralized dentin via micromechanical interlocking, the bond between the adhesive and dentin obtained from the dentin bonding agents used, is believed to be strong enough to resist a moderate amount of occlusal force for some time. Although porosity existed at the hybrid layer, it did not increase after the loading; that is, there was no greater penetration of silver nitrate. However, the forces and movements during mastication are highly complex and factors such as age, gender, bruxism, and bite habits have a significant influence on the forces measured²⁵. Also, the testing machine used could only produce axial cyclic loads, while the movements of mastication in the oral environment are three-dimensional in pattern³¹. Thus, the test conditions simulate but do not duplicate clinical conditions, and this must be considered when interpreting the findings. This study was performed for the quantitative analysis of silver distribution by using SEM Images. However, it might be speculated that TEM can give an informative image for quantitative analysis.

V. CONCLUSION

Durability of long-term dentin adhesion can be influenced by hydrolytic degradation. This study was aimed to evaluate the nanoleakage patterns of two experimental adhesives containing relatively hydrophobic monomers before and after load cycling. From this study, the following results were drawn.

1. The dentin adhesive systems used in this study did not achieve perfect sealing at the adhesive/dentin interface. Silver particles were observed in all the groups at the hybrid

layer.

2. However, silver particles were more sparsely distributed in the ethanol containing adhesive group and the methanol containing group than in the Scotchbond Multi-Purpose group ($p < .0001$).
3. There were no changes in nanoleakage patterns after load cycling.

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국문초록

Load cycling에 따른 소수성 실험용 상아질 접착제의 nanoleakage 양상

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본 연구의 목적은 두 가지 실험용 소수성 상아질 접착제와 전통적인 3-step 상아질 접착제의 nanoleakage 양상을 load cycling 전, 후에 비교하여 상아질 접착제의 내구성을 예측해 보고자 하는 것이다.

두 가지 실험용 소수성 상아질 접착제 즉, 에탄올 포함 상아질 접착제, 메탄올 포함 상아질 접착제를 만들었다. 대구치 30개의 치관부 3분의 1을 절단하고 임의로 3군으로 나누어 각각 Scotchbond Multi-Purpose (3M ESPE, St. Paul, MN, USA), 에탄올 포함 상아질 접착제 및 메탄올 포함 상아질 접착제를 사용하여 상아질 접착 후, 복합레진으로 치관부를 수복하였다. 각각의 접착 시스템을 Load cycling 여부에 따라 두 군으로 나누고 각각의 치아를 접착 계면에 수직으로, 약 2.0 mm의 두께가 되도록 절단하여 치아 마다 2개의 시편을 얻어 각 군 당 10개의 시편을 얻었다. 시편을 50 % ammoniacal silver nitrate를 이용하여 염색한 후 주사전자현미경을 이용하여 혼성층의 nanoleakage를 관찰하였다. 은 침착의 분포는 image analysis software (Scion Image Beta 4.03, Scion Corp., Frederick, MD, USA)를 이용하여 gray value로 계산하였고 이원분산분석법으로 통계처리하였다. 3종의 상아질 접착제에서 모두 nanoleakage 가 관찰되었으나, 에탄올 포함 상아질 접착제와 메탄올 포함 상아질 접착제에서 Scotchbond Multi-Purpose 보다 적은 양의 nanoleakage 가 관찰되었다 ($p < .0001$). 각각의 상아질 접착제에서 load cycling에 따른 nanoleakage 양상의 변화는 관찰되지 않았다.

이상의 결과에서 친수성기를 줄이고 소수성기를 극대화 한 실험용 상아질 접착제가 접착 계면의 내구성 향상에 도움을 줄 수 있을 것으로 사료된다.

주요어: 소수성 상아질 접착제, 내구성, 혼성층, Nanoleakage, Load cycling