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Effect of Surface Treatments and Different Adhesives on the Hybrid Layer Thickness of Non-carious Cervical Lesions

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Clinical Relevance

The quality of the hybrid layer created in non-carious cervical sclerotic lesions may determine the longevity of cervical composite restorations.

SUMMARY

This *in vitro* study observed and compared the resin and non-carious sclerotic dentin interfaces generated by three different adhesives and two different techniques, using a scanning electron microscope (SEM). Thirty-two human premolars, with buccal, non-carious cervical lesions, were used. The teeth were randomly divided into eight groups. Group 1: Sclerotic dentin was treated with Single Bond (3M). Group 2: After superficial sclerotic dentin was removed with a diamond bur (Diatech, Coltene), the dentin surfaces were treated with Single Bond. Group 3: Sclerotic dentin was treated with Clearfil SE Bond (Kuraray). Group 4: After superficial sclerotic dentin was removed with a diamond bur, Clearfil SE Bond was applied. Group 5: Sclerotic dentin was treated with Xeno III (Dentsply). Group 6: After superficial sclerotic dentin was removed with a bur, Xeno III was applied to the dentin surfaces. For Groups 7 and 8, after the superficial sclerotic dentin was removed with a diamond bur, Clearfil SE Bond, with an additional 37% phosphoric acid gel, was used on the sclerotic dentin surfaces in Group 7 and Xeno III was used in Group 8. In all groups, the cavities were restored with Filtek Supreme (3M). All the specimens were sectioned longitudinally and polished along the cut surface. The sections were treated with 37% orthophosphoric acid for five seconds, rinsed with water and treated with 5% NaOCL for 10 minutes. The specimens were then gold-sputter coated and evaluated under SEM. The thick-

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ness of the hybrid layer was measured on the gingival, occlusal and axial dentin interfaces. ANOVA was performed to determine whether there were any statistically significant differences in hybrid layer thickness. Post-hoc multiple comparisons were done with Tukey's test. Hybrid layer thickness was increased with all adhesives when superficial dentin was removed with a bur. Hybrid layer thickness showed significant differences between total-etch and self-etch systems.

INTRODUCTION

A non-carious cervical lesion is the loss of hard dental tissue on the neck of a tooth.[1] These lesions are characterized by a slow, gradual loss of tooth substances, resulting in smooth, wedge-shaped defects along the cervical enamel junction.[2] These unrestored, angular, wedge-shaped lesions demonstrate severe stress concentration that varies with the location of the teeth in the oral cavity. These stresses can be partially relieved after placing a restoration.[3]

Bonding resin composite restorations without cavity preparation are proving to be excellent for restoring teeth with non-carious cervical lesions.[4] Several authors have reported that it is essential to form a hybrid layer at the resin composite dentin interface to obtain proper bonding.[5-7] Dentin adhesives employ two different means to achieve the goal of micromechanical retention between resin and dentin. The first method, the total-etch or etch-rinse technique, attempts to remove the smear layer completely via acid etching and rinsing. The second approach, the self-etch technique, aims at incorporating the smear layer as a bonding substrate.[8] Two-step self-etching primers combine etching and priming into a single step. Recently, single-step self-etch adhesives were introduced, representing a further reduction in bonding steps that eliminate technique sensitivity and practitioner variability, both of which are associated with use of total-etch adhesives.[9]

Sclerotic dentin is an abnormal bonding substrate that exhibits a high degree of variability, both in terms of blocking the dentinal tubules and in thickness of the surface hypermineralized layer. Several reports have indicated that resin bond strengths to non-carious sclerotic cervical dentin are lower than bonds to normal dentin.[10-11] This is thought to be due to tubule blockage by mineral salts, preventing resin tag formation.[3-12] Tay and Pashley[3] advised physical removal of the superficial layers with a bur to improve adhesion to highly sclerotic dentin. However, the formation of a smear layer of acid resistant hypermineralized dentin chips and whitlockite crystals derived from sclerotic dentin may create additional diffusion barriers for both total-etch and self-etch adhesives.[3]

This *in vitro* study observed and compared the resin and non-carious sclerotic dentin interfaces generated by three different adhesives and two different techniques using a scanning electron microscope (SEM).

METHODS AND MATERIALS

Thirty-two human premolars, extracted for periodontal reasons and having buccal non-carious cervical lesions, were stored in saline until they were used in the experiment. All lesions exhibited hard and smooth surfaces and were not carious. The lesions were gently cleaned using a slurry of pumice and water with a slow rotating rubber cup. The specimens were then randomly divided into eight groups (n=4). All the teeth were treated and restored with the materials listed in Table 1 as follows:

Group 1: Sclerotic dentin was etched for 15 seconds with 37% phosphoric acid gel (Vococid,Voco, Cuchaven, Germany), rinsed with water and gently air dried. A total-etch adhesive system (Single Bond, 3M, St Paul, MN, USA) was applied to the etched dentin surfaces, gently air dried and light cured for 20 seconds.

Group 2: After superficial sclerotic dentin was removed with a diamond bur (Diatech, Coltene,

Table 1: *The Composition of Materials Used in This Study*

Material	Manufacturer	Composition
Single Bond (Two-step total-etch adhesive) pH: 5.	3M ESPE, St Paul, MN, USA	**Acid**: 37% phosphoric acid **Adhesive**: Bis-GMA, HEMA, poly-alcenoic copolymer, water, ethanol
Clearfil SE Bond (Two step- self etch adhesive) pH: 2.7	Kuraray, Osaka, Japan	**Primer**: MDP, HEMA, water, hydrophilic dimethacrylate, camphoroquinone, p-toluidine **Adhesive**: Bis-GMA, MDP, HEMA, hydrophilic dimethacrylate, camphoroquinone, p-toluidine, silanated colloidal silica
XENO III (One-step self-etch adhesive) pH: 1.5	Dentsply/DeTrey, Kontanz, Germany	**Primer**: HEMA, water, ethanol, BHT, stabilizer, nanofiller **Adhesive**: PEM-F, UDMA, camphoroquinone, EPD
Filtek Supreme (Nanofill composite)	3M ESPE, St Paul, MN, USA	Bis-GMA, Bis-EMA, UDMA, TEGDMA, nanosilica filler, zirconi-silica nano cluster

Whaledent AG, Switzerland), 37% phosphoric acid jel was applied for 15 seconds, rinsed with water and gently air dried. The etched dentin surfaces were then treated in the same manner as Group 1 (Single Bond).

Group 3: A self-etch primer (Clearfil SE Primer, Kuraray, Osaka, Japan) was applied on the sclerotic dentin for 20 seconds and gently air dried. A self-etch adhesive (Clearfil SE Bond, Kuraray, Osaka, Japan) was applied, air thinned and light cured for 10 seconds.

Group 4: After superficial sclerotic dentin was removed with a diamond bur, Clearfil SE Bond was applied in the same manner as Group 3.

Group 5: Self-etch primer and bond (Xeno III, Dentsply, Kontanz, Germany) was mixed and applied to the sclerotic dentin surfaces for 10 seconds. After being air thinned, the adhesive resin was light cured for 10 seconds.

Group 6: After superficial sclerotic dentin was removed with a diamond bur, Xeno III was applied to the dentin surfaces as explained in Group 5.

Group 7: After superficial sclerotic dentin was removed with a diamond bur, 37% phosphoric acid gel was applied for 15 seconds, rinsed with water and gently air dried. Self-etching primer (Clearfil SE Primer) was applied to the sclerotic dentin for 20 seconds and gently air dried. Adhesive resin (Clearfil SE Bond) was applied, air thinned and light cured for 10 seconds.

Group 8: After superficial sclerotic dentin was removed with a diamond bur, 37% phosphoric acid gel was applied for 15 seconds, rinsed with water and gently air dried. Xeno III was applied to the sclerotic dentin for 10 seconds, air thinned and light cured for 10 seconds.

In all groups, the cavities were restored with a nanofill resin composite (Filtek Supreme, 3M) in bulk and light cured with a halogen lamp (Hılux, Benlioglu Dental Inc, Ankara, Turkey) with an intensity of 620 mW/cm^2 for 40 seconds. All specimens were stored in tap water at 37°C for 24 hours and sectioned longitudinally through the lesion using a slow-speed diamond saw under copious water. Each of the cross sections was polished along the cut surface with a series of wet silicon carbide disks (#600, 800, 1000, 1200) and rinsed under water. The sections were treated with 37% orthophosphoric acid for five seconds, rinsed with water and treated with 5% NaOCl for 10 minutes. The samples were then left at room temperature for desiccation and gold-sputter coated for evaluation under SEM. The thickness of the hybrid layer was measured on the gingival, occlusal and axial dentin interfaces. Five measurements were performed on each specimen and the mean of the hybrid layer thickness were calculated. ANOVA was performed to determine whether there were any statistically significant differences in hybrid layer thickness. Post-hoc multiple comparisons were made with the Tukey and Tamhane tests.

RESULTS

Table 2 shows the thickness of the hybrid layer for each material on the gingival, occlusal and axial dentin of non-carious cervical lesions.

The SEM images of the resin-dentin interfaces of the adhesive systems are shown in Figures 1 through 8. The hybrid layer could be observed in all specimens. The thickness of the hybrid layer varied, depending on the bonding system and the dentin substrate (Tables 2 and 3).

A hybrid layer and resin tags were clearly seen, especially in the gingival and axial dentin regions (Figures 2, 4, 5 and 6). When the dentin regions were compared, all the adhesives showed thinner hybrid layers at the occlusal dentin region. There were no resin tags in all groups at the occlusal dentin region (Figures 1 through 8).

In Group 2, resin penetration into dentinal tubules or resin tags were clearly observed in the gingival and axial dentin regions after bur preparation. Resin tags were noticeable and thick (Figures 2 and 3).

Table 2: *Mean and SD of Hybrid Layer Thickness of Adhesives in Non-carious Cervical Lesions*

Groups	Gingival	Axial	Occlusal
Group 1 (Single Bond without bur)	2.6 ± 0.336^{aA}	2.4 ± 0.402^{aAB}	1.5 ± 0.553^{abcdeAC}
Group 2 (Single Bond with bur)	4.04 ± 0.466^{bA}	3.3 ± 0.540^{aB}	2.2 ± 0.406^{aC}
Group 3 (Clearfil SE without bur)	1.3 ± 1.546^{cA}	0.6 ± 0.158^{bB}	0.5 ± 0.955^{bC}
Group 4 (Clearfil SE with bur)	2.8 ± 1.207^{abcdA}	2.9 ± 0.465^{aA}	1.1 ± 0.343^{cB}
Group 5 (Xeno III without bur)	0.8 ± 0.080^{dA}	0.8 ± 0.107^{bA}	0.4 ± 0.086^{bB}
Group 6 (Xeno III with bur)	2.7 ± 0.398^{abA}	2.2 ± 0.230^{aA}	1.2 ± 0.231^{dB}
Group 7 (37% phosphoric acid+ Clearfil SE with bur)	4.8 ± 0.801^{bA}	4.5 ± 0.961^{aB}	2.5 ± 0.461^{eC}
Group 8 (37% phosphoric acid + Xeno III with bur)	3.3 ± 0.593^{abA}	3.0 ± 0.499^{aA}	2.4 ± 0.320^{eB}

Lower cases represent the significant differences between the materials (p<0.05), capitals represent the significant differences between the dentin regions (gingival, axial, occlusal) (p<0.05).

Table 3: *Tamhane Test Results of Multiple Comparisons of Experimental Groups*

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
Group 1 (Single bond without bur)	-	0.045*	0.000*	1.000	0.000*	1.000	0.003*	0.103
Group 2 (Single Bond with bur)	0.045*	-	0.000*	0.476	0.000*	0.019*	0.863	1.000
Group 3 (Clearfil SE without bur)	0.000*	0.000*	-	0.003*	1.000	0.000*	0.000*	0.000*
Group 4 (Clearfil SE with bur)	1.000	0.476	0.003*	-	0.002*	1.000	0.022*	0.888
Group 5 (Xeno III without bur)	0.000*	0.000*	1.000	0.002*	-	0.000*	0.000*	0.000*
Group 6 (Xeno III with bur)	1.000	0.019*	0.000*	1.000	0.000*	-	0.002*	0.041*
Group 7 (phosphoric acid+ Clearfil SE with bur)	0.003*	0.863	0.000*	0.022*	0.000*	0.002*	-	0.245
Group 8 (phosphoric acid + Xeno III with bur)	0.103	1.000	0.000*	0.888	0.000*	0.041*	0.245	-

**represent the significant differences between the groups (p<0.05).*

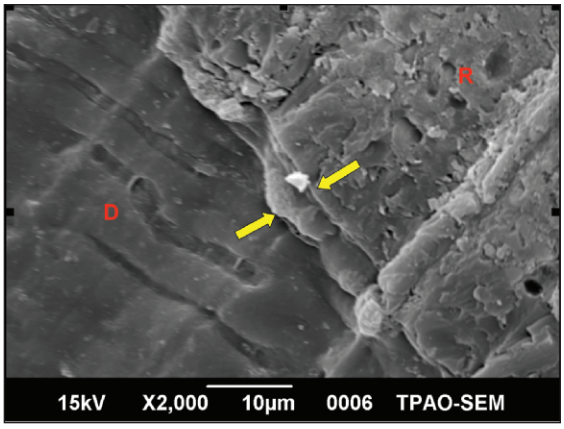

Figure 1. *SEM micrograph (2000x) of a sample. The occlusal dentin region was treated with Single Bond. Resin penetration into the dentinal tubules cannot be identified. A 3.75 µm thick hybrid layer was observed (the arrows). R: resin, D: dentin.*

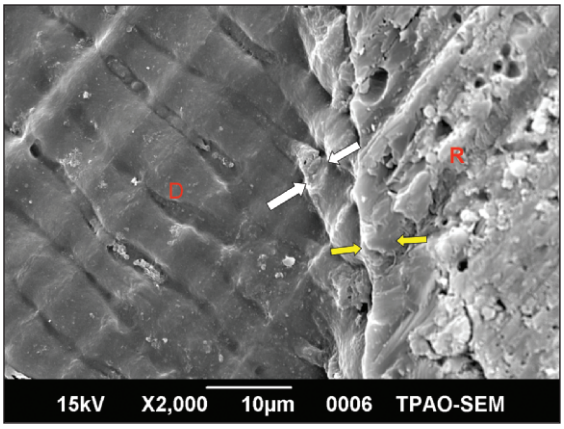

Figure 2. *SEM micrograph (2000x) of the axial dentin region of non-carious sclerotic dentin. In this sample, Single Bond adhesive was used after bur removal. Funnel-shaped, short resin tags through the dentin tubules (white arrow) and hybrid layer (yellow arrows) can clearly be identified. R: resin, D: dentin.*

In specimens where a bur was used before Clearfil SE Bond, the hybrid layer and minimal tag formation was observed (Figure 4).

When Xeno III was applied, resin tags and their lateral branches were more scattered and thinner compared to the others (Figures 5 and 6).

When only a bur was used to remove the superficial layer (Groups 2, 4 and 6), the hybrid layer thickness significantly increased with all the tested adhesive systems ($p<0.05$) (Table 2).

Group 1 (total-etch adhesive) was significantly different from Group 3 (two-step self-etch adhesive) and Group 5 (one-step self-etch adhesive) ($p<0.05$). Groups 3 and 5 did not show any significant difference ($p>0.05$) (Table 2).

By using a diamond bur with an additional 37% phosphoric acid etching before a self-etch primer, the hybrid layer thickness was increased significantly ($p>0.05$) (Group 7 and 8) (Figures 7 and 8).

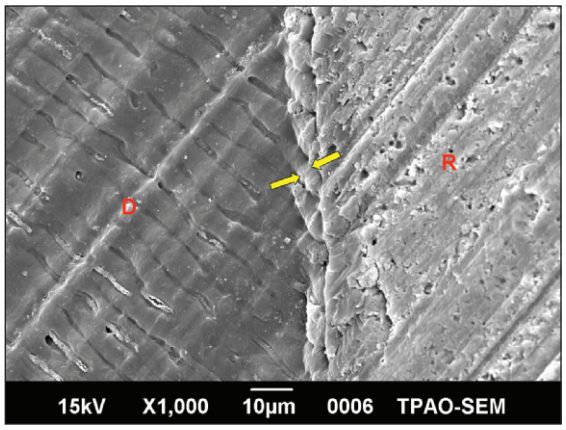

Figure 3. *SEM micrograph (1000x) of the axial dentin region of non-carious sclerotic dentin. In this sample, Single Bond was used after bur removal. Long and thin resin tags could be identified (arrows). R.resin, D: dentin.*

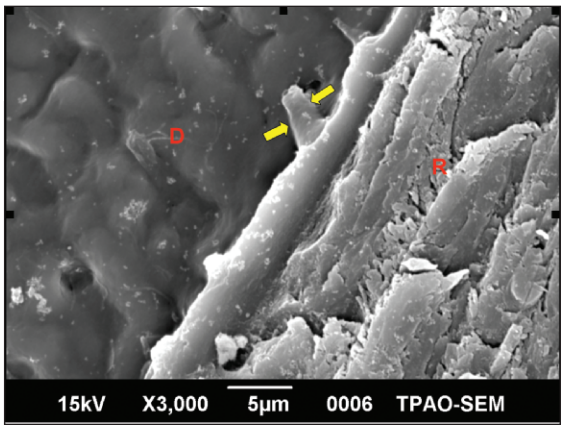

Figure 4. *SEM micrograph (3000x) of the gingival dentin region of a non-carious sclerotic lesion. Clearfil SE bond was used after removal of the superficial layer with a bur. Wide and short resin tags are observed with a flat end (arrow). D: dentin, R: resin.*

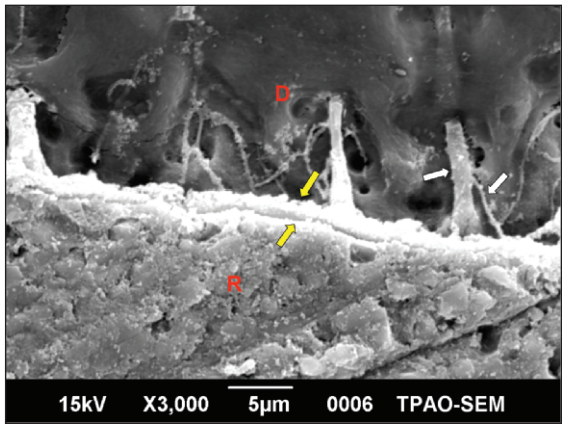

Figure 5. *SEM micrograph (3000x) of the gingival dentin region of a non-carious cervical lesion. Xeno III was used in this sample before composite restoration. Thin and cylindirically-shaped resin tags and their lateral branches are clearly identified (white arrows). Yellow arrows show the hybrid layer. R: resin, D: dentin.*

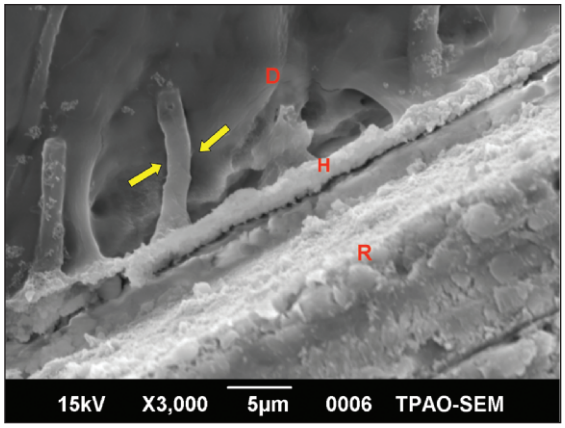

Figure 6. *SEM micrograph (3000x) of the axial dentin of non-carious sclerotic dentin treated with Xeno III. Cylindirical tag formation is clearly observed (arrows). R: resin, D: dentin, H. hybrid layer.*

DISCUSSION

It has been suggested that the essential mechanism of adhesion between dentin bonding systems and the dentin substrate is the formation of a hybrid layer composed of resin that has been impregnated into the superficial decalcified dentin surface.[13]

A hybrid layer is formed via two techniques, namely total-etch and self-etch.[14] In the total-etch technique, 37% phosphoric acid is used to remove the smear layer and demineralize the underlying dentin.[14] In addition to demineralizing intertubular and peritubular dentin, acids open the dentin tubules and expose a dense filigree of collagen fibers, thus increasing the microporosity of the intertubular dentin.[14] In the self-etch technique, another type of acidic conditioner, including a phosphonated resin molecule, is used, which simultaneously performs two functions—etching and priming of the dentin and enamel.[13] Self-etch systems can be classified according to their application steps as one-step or two-step and, according to their pH values, they can be classified as mild, moderate or strong.[13] Clearfil SE Bond is a mild self-etch adhesive with a pH value of 2.7. Xeno III is involved in moderate self-etch adhesives, with a pH value of 1.5.

Sclerotic dentin is an abnormal bonding substrate that exhibits a high degree of variability, both in terms of blocking the dentinal tubules and thickness of the surface hypermineralized layer.[3] Irrespective of the use of a total-etch or a self-etch technique, bonding to pathologically altered substrates, such as sclerotic dentin from non-carious cervical lesions, generally leads to compromised bonding.[3] Reduced bonding efficacy was attributed to a combination of factors, including the oblitera-

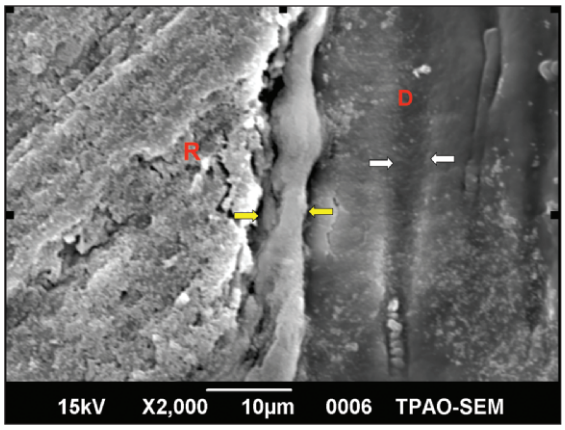

Figure 7. *SEM micrograph (2000x) of the axial dentin region. Clearfil SE Bond and phosphoric acid were used after bur removal. The thickest hybrid layer was seen in this group (approximately 4.5 µm, yellow arrows). Dentin tubules are parallel to the cavity surface (white arrows). D: dentin, R: resin.*

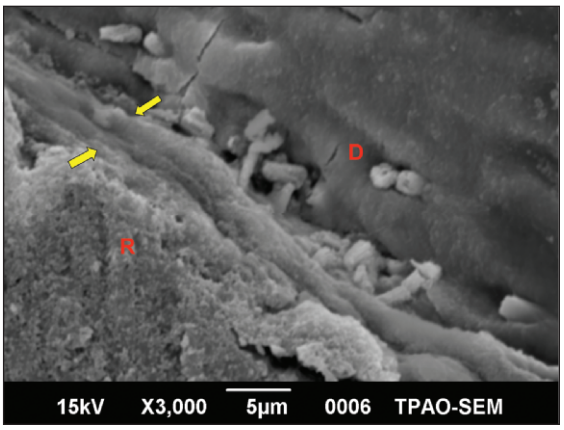

Figure 8. *SEM micrograph (3000x) of the occlusal dentin region. Clearfil SE Bond was used with additional phosphoric acid after bur removal. R: resin D: dentin.*

tion of dentinal tubules with sclerotic casts and the presence of an acid resistant hypermineralized layer.[3]

In the current study, all of the adhesives formed a hybrid layer in all parts of the sclerotic lesions. The hybrid layer thickness of sclerotic lesions formed with total-etch adhesives was significantly thicker than the hybrid layer formed with two self-etch adhesives ($p<0.05$).

Tay and others[3] reported that, when a self-etch adhesive was applied to sclerotic dentin with a thick hypermineralized layer (>0.5 µm), the self-etch primer was not able to etch this layer and did not create a hybrid layer. Arrais and others[15] evaluated the morphology and thickness of the resin infiltrated dentin layer after application of the adhesive systems and measured the thickness of the hybrid layer on normal dentin for Clearfil SE Bond at 1.22 ± 0.4. Sakoolnamarka and others[16] reported that Clearfil SE Bond created a very thin hybrid layer on non-carious cervical lesions with few resin tags (approximately 0.3 µm in the axial wall of the lesion). In the current study, the thickness of the hybrid layer, created by Clearfil SE Bond with sclerotic dentin, appeared to be thicker than what was found in the former study (approximately 0.6 µm in the axial wall of the lesion). Although the same material and technique was used, the difference in the degree of dentin sclerosis of cervical lesions might have an effect on the different results.

Xeno III is a two-component, one-step self-etch adhesive. Its solvent includes a combination of water, ethanol and nanofillers. The degree of demineralization and interaction with dentin correlates with the acidity of self-etch adhesives, as observed in previous investigations.[10,11,17] In the current study, a moderate self-etch adhesive, Xeno III (pH: 1.5), and a mild self-etch adhesive, Clearfil SE (pH: 2.7), created similar hybrid layer thicknesses ($p<0.05$) (Table 2). Xeno III created thin, infrequent resin tags in the gingival dentin of non-carious cervical lesions (Figure 5).

Harnirattisai and others[18] observed the interface between a total-etch adhesive and the dentin walls of cervical erosion/abrasion lesions. After 37% phosphoric acid gel etching for 60 seconds, resin impregnated intertubular dentin with a thickness range of between 0.3 to 3 µm occurred. At the gingival wall of the cervical lesions, a hybrid layer thickness was found to be in the range of 1-3 µm, while at the occlusal wall, the thickness was within the 0.3-2 µm range.[18] In the current study, similar results were observed with the total-etch adhesive (Singe Bond) in non-carious cervical lesions, although 37% phosphoric acid gel was applied for 15 seconds. (Figure 1).

Nakornchai and others[19] reported that the hybrid layer thickness of Single Bond adhesive in intact and caries-affected dentin was approximately 2-3 µm. In the same study, Clearfil SE Bond created approximately a 1 µm thick hybrid layer. Similar to this study, the resin tags of the total-etch adhesive group in intact and caries-effected dentin had a funnel shape (Figures 2 and 3) (resulting from removal of the peritubular dentin), while resin tags of the self-etching adhesive group exhibited a cylindrical shape[19] (Figure 5).

Although most tubules were obliterated, the direction of the tubules seemed to play an important role in demineralization of superficial dentin. In all groups, the occlusal wall, where dentinal tubules run parallel to the lesion surface, exhibited thinner hybrid layers than the gingival and axial walls. However, only in Group 1 ($p<0.05$) was this difference not significant.

It has been suggested that the strength of an interfacial bond has a 20% contribution from resin infiltration,

which is derived from resin tag formation, and another 20%, which is derived from hybridization of the intertubular dentin.[20] In the current study, when adhesives were used without bur preparation, Clearfil SE Bond was not able to create resin tags in all dentin regions of non-carious sclerotic lesions. However, in specimens where the superficial dentin was removed with a bur before the application of Clearfil SE primer, the adhesive created few resin tags in the gingival and axial dentin regions (Figure 4). Because of the tubule direction in the occlusal dentin region, resin tags were not observed with all adhesives.

Vaysman and others observed that the highest reduction in bonded dentin permeability or an increase in sealing ability occurred when the self-etch adhesive system was applied to the roughest dentin surface. These authors stated that high surface roughness might have increased the dentin surface, allowing for better contact between the adhesive and dental substrate.

Removal of the superficial layer of sclerotic dentin with a bur and moving the bonding interface pulpward has been suggested to improve intertubular retention.[3] In the current study, the hybrid layer thickness increased when the surface hypermineralized dentin was removed with a bur before applying the adhesives ($p<0.05$) (Table 2). Some researchers have stated that the formation of a smear layer that contains acid-resistant hypermineralized dentin chips and whitlockite crystals derived from sclerotic casts may create an additional diffusion barrier for self-etch and total-etch adhesives.[3] However, the results of the current study indicate that self-etch and total-etch systems can remove or pass through the smear layer and create a hybrid layer. When a diamond bur and 37% phosphoric acid were used before using the self-etch adhesives, the hybrid layer thickness was increased.

The current study is limited to measurements of the hybrid layer thickness using different materials and techniques in sclerotic dentin surfaces, although it does not go into depth relating bond strength, longevity or stress measurements. However, based on these results, it is clear that hybrid layer thickness was effected by different adhesives and techniques. Additionally, hybrid layer thickness was increased when sclerotic dentin was prepared with a bur.

CONCLUSIONS

1. In the current study, hybrid layer thickness showed a significant difference between total-etch and self-etch systems in non-carious sclerotic lesions without removal of the superficial layer.
2. One-step and two-step self-etch adhesives did not show differences, regardless of the presence of the superficial layer.
3. Hybrid layer thickness was increased for all adhesives when superficial dentin was removed with a bur.
4. An additional 37% phosphoric acid application with self-etch adhesives significantly increased the thickness of the hybrid layer in specimens prepared with a diamond bur.
5. All adhesives showed the thinnest hybrid layer in the occlusal dentin region.

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