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Sealing Ability of Dentin Adhesives/Desensitizer

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Clinical Relevance

Depending on the application of the individual adhesives used, dentin adhesives seal dentin surfaces and reduce dentinal permeability. Dentin adhesives possessing a high sealing ability for dentin surfaces are suggested for the treatment of dentin hypersensitivity.

SUMMARY

Objectives: This *in vitro* study evaluated the sealing ability of dentin adhesives and a desensitizer. Methods: Standardized Class V cavities were prepared on the buccal and lingual surfaces of 55 freshly extracted human molars. The teeth were cut into two sections in a mesio-distal direction. All specimens were randomly divided into 11 groups (n=10). In each group, the cavities were treated with one of eight different dentin adhesives, a dentin desensitizer or a special combination of these, except for the control group. After all the cavities had been stained for 24 hours with 0.5% methlyene blue solution, the teeth were longitudinally cut into two sections through the center of the cavities. Dye penetration was recorded according to the stained areas. Epoxy resin replicas of two specimens per group were analyzed by SEM. Results: All dentin adhesives/desensitizer significantly reduced dentinal permeability (*p*<0.05). The sealing ability of the different dentin adhesives/desensitizer was significantly different (*p*<0.001). The degree of dye penetration corresponded well with the surface morphology of the dentin surfaces after the various treatments. Conclusions: None of the dentin adhesives/desensitizer could completely block fluid percolation through the dentinal tubules, but current dentin adhesives/desensitizers can significantly reduce dentin permeability.

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INTRODUCTION

Based on the hydrodynamic theory, extrinsic stimuli induce fluid movement through the dentinal tubules and evoke dental hypersensitive activity.[1-2] If the extrinsic stimuli are mechanically blocked by dentinal tubular occlusion, hypersensitivity can be relieved.[1-2] Dentin adhesives can cause tubular occlusion of the sensitive dentinal surfaces.[3] Therefore, the topical application of dentin adhesives is an effective way to occlude the patent dentinal tubules of cervical lesions.[4]

Recently, dentin adhesives have been developed to simplify the three cardinal clinical steps (etching, priming and bonding of tooth substrates) into two steps or one step. However, one-step self-etching adhesives were found to be permeable to fluid.[5-6] Little is known about whether hydrophilic self-etching primers covered with a more hydrophobic bonding resin can completely seal the underlying hydrated dentin.[7] Some data indicate that incomplete micro-emulsion polymerization of the hydrophilic primers and hydrophobic bonding resin affect the sealing ability of dentin adhesives on dentin surfaces.[7]

This *in vitro* study 1) evaluated the sealing ability of a dentin desensitizer and one-, two- and three-step adhesives on dentin surfaces in shallow Class V cavities and 2) it investigated the surface morphology of dentin surfaces treated with dentin adhesives/desensitizer using scanning electron microscopy.

The hypothesis tested in this study was that the topical application of dentin adhesives on the dentin surface could reduce the exogenous fluid penetration into the dentinal tubules but not completely block it.

METHODS AND MATERIALS

Fifty-five freshly extracted, impacted or erupted human molars, without visible caries, restorations and defects, stored in tap water within one month, were used to study the sealing ability of the dentin adhesives/desensitizer (Table 1). Standardized Class V cavities (mesio-distal dimension = 2.5 mm, gingivo-coronal dimension = 3 mm, depth = 2 mm) located at the cementoenamel junction (CEJ) were prepared on the buccal and lingual surfaces of the teeth using a carbide bur (SSW FG559, Lakewood, NJ, USA) at high speed under water cooling. The teeth were longitudinally cut into two sections in a mesio-distal direction. All the specimens were randomly assigned to 11 groups of 10 specimens each.

The bur-prepared cavities were left without any treatment in the control group (Group I). In Group II, the cavities were rubbed with Gluma Desensitizer prime & protect (GD) for 60 seconds and gently blown with oil-free compressed air for five seconds. In Group III, the cavities were treated, as in Group II, and subsequently, Gluma Comfort Bond (GDCB) was applied to the primed cavities, gently blown for five seconds and light-cured for 20 seconds. In Group IV, Resulcin AquaPrime (Resulcin AquaPrime + MonoBond, APMB) was applied to the cavities for 60 seconds, gently blown for five seconds, and subsequently, MonoBond was applied, gently blown, then light-cured for 20 seconds. In Groups V and VI, a mixture of Clearfil Liner Bond 2v Primer A and B was applied to the cavities for 60 seconds, gently blown for five seconds and, subsequently, Bond A (CLB A–Group V) or a mixture of Bond A and B (CLB AB–Group VI) was applied to the primed cavities, gently blown again, then light-cured for 20 seconds, respectively. In Group VII, the cavities were etched with 37% phosphoric acid for 15 seconds, rinsed with water spray for 20 seconds, primed (Scotchbond MP, SBMP) for 60 seconds, gently blown for 5 seconds, treated with adhesive, gently blown again and light-cured for 10 seconds. In Group VIII, a mixture of Ecusit Primer A+ B was applied to the cavities for 60 seconds, gently blown for five seconds, and, subsequently, Ecusit Mono (Ecusit Primer + Mono, Ecusit) was applied to the cavities, gently blown again and light-cured for 20 seconds. Prompt L-Pop (Prompt, Group IX) or Voco Solobond M (SB M, Group X) was applied to the cavities for 60 seconds, gently blown for 5 seconds and light-cured for 20 seconds, respectively. In Group IX, the Prompt L-pop application procedure was repeated once. In Group XI, Solid Bond P Ethanol was applied to the cavities for 60 seconds, gently blown and, subsequently, Solid Bond S (Solid Bond experimental, SB exp) was applied, gently dried and light-cured for 20 seconds.

Table 1 summarizes the treatment methods, the dentin desensitizer/adhesives used in this laboratory study and the corresponding codes for the dentin desensitizer/adhesives and control group. The dentin adhesives/desensitizer were strictly applied according to the manufacturer's instruction except for the special experimental procedures in Groups III and VI. For systems requiring light curing, an Ortholux XT visible light-curing unit (3M Unitek) was used. The curing unit was held at a distance of 5 mm above the cavities.

SEM Observations

Two specimens per group were randomly selected to make impressions with an addition-type polyvinylsiloxane (President, Coltene AG, Altstatten, Switzerland, Lot: MH892) and to produce epoxy resin (Stylcast 1266, Stycast, T-E-Klebetechnick, Hannover, Germany) replica models. Subsequently, the replicas were mounted on aluminum stubs, gold-sputtered and analyzed with field-emission environmental scanning electron microscopy (FEI XL30 ESEM FEG, FEI Philips Company, Eindhoven, Holland).

Dye Penetration

After all the specimens were treated as mentioned above, the teeth were kept in a closed, 100% humidity box, with the cavity opening directed upward. Subsequently, a solution of 0.5% methlyene blue was slowly injected into the prepared cavities to about 0.5 mm under the cavosurface margins. After 24 hours, the remaining dye in the specimens' cavities was thoroughly absorbed with small cotton pellets. The teeth were longitudinally cut into two sections through the center of the cavities in a mesio-distal direction. The dye penetration scores (0-4) were recorded with a stereomicroscope at 10-fold magnification according to the stained

Table 1: *Materials Used in This Laboratory Study and Mode of Application*

Groups	Dental Bonding Agents (manufacturers)	Solvents of Primers	Hydrophobic Bonding Resin	Steps of Application	# of Steps	Code
I	No bonding agent	-	-	No treatment	none	Ctr
II	Gluma Desensitizer prime & protect (Heraeus Kulzer, Dormagen, Germany, Lot #DAEXZI 01 99)	Water	-	Apply to the dentin for 60 seconds, gently blow for 5 seconds	one	GD
III	Gluma Desensitizer prime & protect + Gluma Comfort Bond (Heraeus Kulzer D-41538 Dormagen, Germany, Lot #020022)	Water	+	Apply Gluma Desensitizer to the cavities for 60 seconds, gently blow for 5 seconds, apply Gluma Comfort Bond, gently blow, light cure for 20 seconds.	two	GDCB
IV	Resulcin AquaPrime + MonoBond (Merz+CO, Lütjenburg, Germany, Lot #98150069)	Water	+	Mix AquaPrime A with water (1:1), apply to the cavities for 30 seconds, gently air dry, apply MonoBond, gently blow, light cure for 20 seconds.	two	APMB
V	Clearfil Liner Bond 2v (Primer A+B, Bond A) (Kuraray Co, Osaka, Japan, Lot #41112)	Mixture of water & ethanol	+	Mix Primer A and B, apply to the cavities for 30 seconds, gently blow, apply Bond A, gently blow, light cure for 20 seconds.	two	CLB A
VI	Clearfil Liner Bond 2v (Primer A+B, Bond A+B)	Mixture of water & ethanol	+	Mix Primer A and B, apply to the cavities for 30 seconds, air blow gently, apply the mixture of Bond A and B, gently blow, light cure for 20 seconds.	two	CLB AB
VII	Scotchbond MP (3M, St Paul, USA, Lot #19960222)	Mixture of water & ethanol	+	Acid etch for 15 seconds, water spray for 20 seconds, apply a primer for 60 seconds, gently blow, apply adhesive, gently blow, light cure for 10 seconds.	three	SBMP
VIII	Ecusit-Primer A & B + Mono (DMG, Hamburg, Germany)	Water	+	Mix Primer A & B, apply to the cavities for 30 seconds, gently blow, apply Mono, gently blow, light cure for 20 seconds.	two	Ecusit
IX	Prompt L-Pop (ESPE, Seefeld, Germany, Lot #FW005241)	Water	-	Scrub into the cavities for 30 seconds, leave undisturbed for 30 seconds, light cure for 20 seconds. The same treatment for two times.	two	Prompt
X	Solobond M (Voco, Cuxhaven, Germany, Lot #92645)	Acetone	-	Apply to the cavities for 60 seconds, gently blow, light cure for 20 seconds.	one	SB M
XI	Solid Bond experimental (Solid Bond P Ethanol, Lot #21c, Solid Bond S, Lot #30, Heraeus Kulzer, Dormagen, Germany)	Mixture of water & ethanol	+	Apply Solid Bond P Ethanol for 60 seconds, gently blow, apply Solid Bond S, gently dry, light light cure for 20 seconds.	two	SB exp

-: No application of hydrophobic bonding resin. +: Application of hydrophobic bonding resin.

ranges as follows: 0: no dye penetration through the cavity floor into the underlying dentin, 1: dye penetration through less than one-fourth of the cavity floor into the underlying dentin up to one-fourth of the distance between the cavity floor and the underlying wall of the pulp chamber, 2: dye penetration through less than half of the cavity floor into the underlying dentin up to half the distance between the cavity floor and the underlying wall of the pulp chamber, 3: dye penetration through less than three-fourths of the cavity floor into the underlying dentin up to three-fourths of the distance between the cavity floor and the underlying wall of the pulp chamber, 4: dye penetration through more than three-fourths of the cavity floor into the underlying dentin. The maximum dye penetration score in each specimen was recorded instead of the mean score. The Kruskal-Wallis H-test (SPSS Software, version 11.0, SPSS Inc, Chicago, IL, USA) was followed by the Nemenyi Test for multiple comparisons.

RESULTS

SEM Observations

The untreated dentin surface revealed the typical smear layer (Figure 1). The surface morphology of the dentin treated with dentin adhesives greatly varied, depending on the different agents used (Figure 2a-j). The surface morphology of GD-primed dentin was similar to the dentin smear layer (Figure 2a). The GDCB-

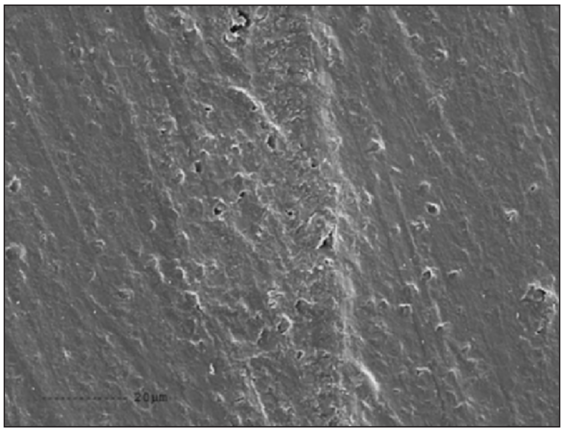

Figure 1: *Smear layer on the dentin surface. The bur-prepared dentin surface revealed a typical smear layer on the dentin surface (1000x, dotted line: 20 µm).*

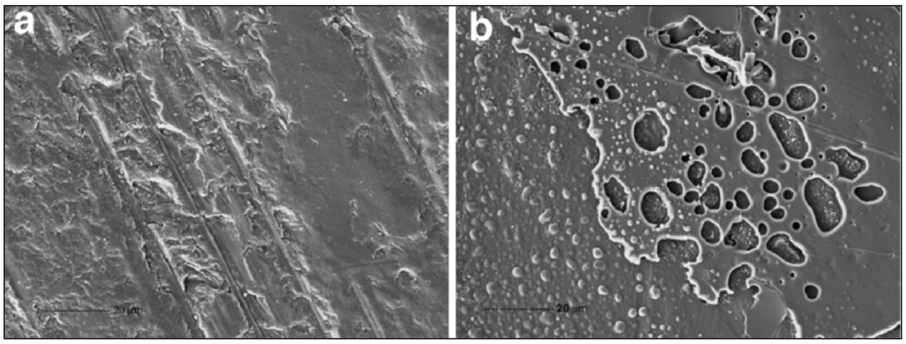

Figure 2: *Dentin surfaces treated with the various dentin adhesives/desensitizer.* Figure 2a*: GD-treated dentin surfaces revealed a dentinal surface pattern similar to the dentin smear layer (1000x, dotted line: 20 µm).* Figure 2b*: GDCB-treated dentin surfaces revealed an incompletely co-polymerized layer on some surface areas. There are many round deposits on the dentin surface as well as porosities of the dentin-bonding layer (1000x, dotted line: 20 µm).*

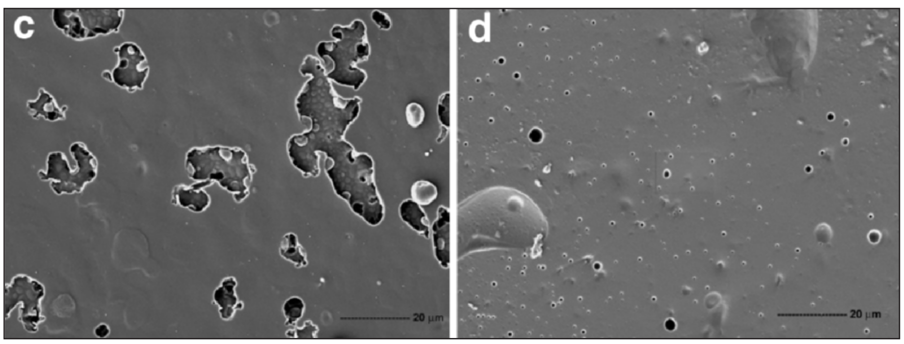

Figure 2c*: APMB-treated dentin surface revealed several big irregular porosities on the dense dentin bonding layer, as well as dentin exposed through porosities of the bonding layer in some areas (1000x, dotted line: 20 µm).* Figure 2d*: The dentin surfaces treated with Primer A+B and Bond A (CLB A) revealed small porosities on most dentin surface areas (1000x, dotted line: 20 µm).*

treated dentin surfaces showed incomplete emulsion co-polymerization of the hydrophilic primer and the hydrophobic bonding resin (Figure 2b). Many semi-sphericities are visible on the dentin surface through disruption of the bonding layer and on the surface of the bonding layer (Figure 2b). The APMB-treated dentin surfaces revealed a smooth, dense dentin-bonding layer dotted with irregular porosities exposed to the dentin surfaces (Figure 2c). The dentin surfaces treated with Primer A+B and Bond A (CLB A) revealed many tiny porosities in the dentin bonding layer (Figure 3d). However, the dentin surfaces treated with primer A + B and Bond A + B (CLB AB) showed a dense bonding layer without any visible dentinal structures (Figure 2e). The surface morphology of the SBMP-treated dentin revealed that some porosities were dotted on the bonding layer, which was still dense at high magnification (Figure 2f). Some tiny particles sparsely deposited onto the dense bonding layer were detected on the Ecusit-treated dentin (Figure 2g). There were considerable porosities in the dense bonding layer of the Prompt-treated dentin (Figure 2h). Many sphericities were detected in the dense dentin-bonding layer of the SBM-treated dentin (Figure 2i). The SB exp-treated dentin surfaces revealed many nanometer-sized porosities in the dentin-bonding layer (Figure 2j).

Dye Penetration Scores

The maximum dye penetration scores observed in Groups I-XI are shown in Table 2. The statistical analysis of the results from pairwise comparisons is shown in Table 3. Compared to the control group, all dentin adhesives/desensitizer significantly reduced dentinal dye permeability ($p<0.05$, Table 3). The sealing ability of the various dentin adhesives was significantly different ($p<0.001$, Table 2). The adhesive Voco Solobond M was far better for the reduction of dentinal permeability than the other dentin adhesives ($p<0.01$) except for Group VI (CLB AB). CLB AB reduced dentinal permeability far better than the other dentin adhesives/desensitizer ($p<0.05$) except for Ecusit. Ecusit was similar to SB exp in terms of reduction of dentinal permeability ($p>0.05$); however, it reduced dentinal permeability far better than APMB, SBMP, Prompt, GDCB and GD. Concerning the reduction of dentinal permeability, SB exp performed similarly to APMB ($p>0.05$), but both performed better than SBMP, Prompt, GBCB and GB ($p<0.05$). Prompt reduced dentinal permeability better than GD ($p<0.05$) but not as well as GDCB ($p>0.05$).

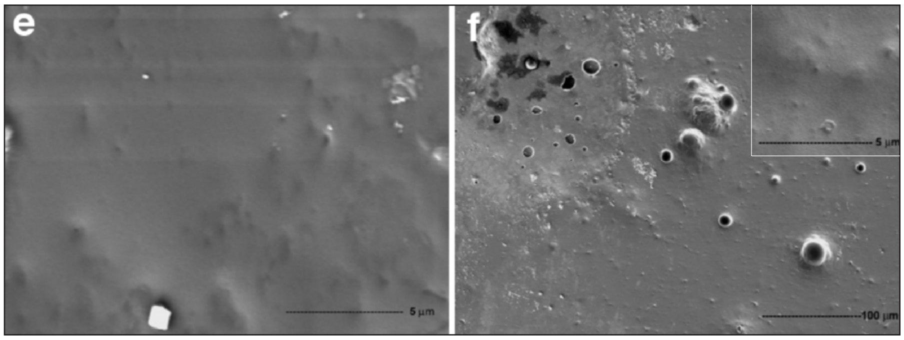

Figure 2e*: The dentin surface treated with primer A + B and Bond A + B (CLB AB) revealed a dense bonding layer without any dentinal structures observed (10,000x, dotted line: 5 µm).* Figure 2f*: The SBMP-treated dentin surface revealed some porosities on some bonding layer areas (210x, dotted line: 100 µm), as well as a dense bonding layer among the porosities at high magnification (5000x, dotted line: 5 µm).*

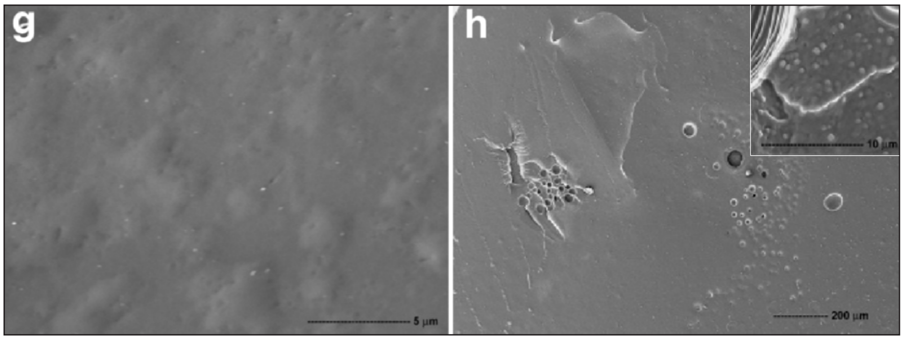

Figure 2g*: Ecusit-treated dentin surface revealed deposits of small particles on the dense dentin bonding layer (5000x, dotted line: 5 µm).* Figure 2h*: Prompt-treated dentin surface revealed relatively large porosities on some areas of the dentin bonding layer (55x, dotted line: 200 µm). The dentin surrounding the porosities was covered by a dentin-bonding layer and the dentin hydroxyapatite became indistinct (2500x, the dotted line: 10 µm).*

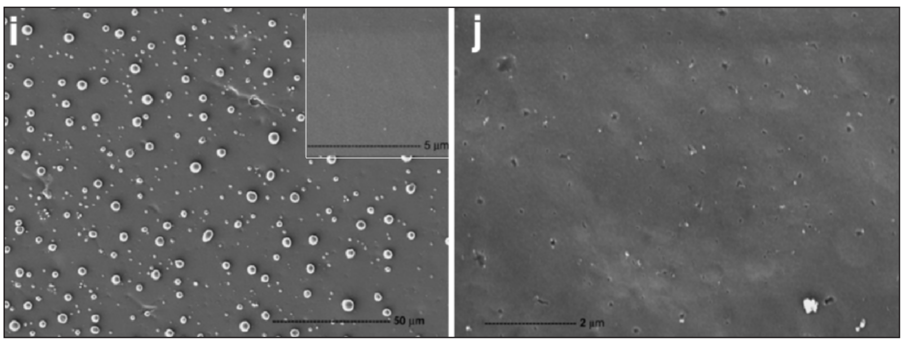

Figure 2i*: The SB M-treated dentin surfaces revealed many variable-sized deposits in dentin bonding layer on the most dentin surface areas (500x, dotted line: 50 µm), and a dense bonding layer among the deposits (5000x, dotted line: 5 µm).* Figure 2j*: The SB exp-treated dentin surfaces revealed nanometer-sized porosities (10,000x, dotted line: 2 µm).*

DISCUSSION

Fluid percolation through the dentinal tubules evokes the intradental nerve activity associated with dentin hypersensitivity.[8-9] Dentin adhesives/desensitizer could be used to mechanically block patent dentinal tubules[10] or physiologically decrease excitability of the intradental nerve[11-12] to relieve hypersensitivity. Actually, it is nearly impossible to completely relieve hypersensitivity in all clinical cases. The findings of this study revealed that the dentin adhesives/desensitizer tested could not completely block dentinal permeability. This finding is consistent with the clinical experience and previous reports about the fluid permeability of current adhesives.[5-7,13-16] Adhesives appear incapable of sealing the restoration and, thus, preventing microleakage.[17] One reason might be that the bonding layer on the dentin surface is disrupted by the burst of blisters or porosities, as shown in Figures 2b and 2c. Furthermore, Hashimoto and others[14] detected fluid movement across the resin-dentin interface during air-drying and during/after polymerization; they also found that adhesive resin films per se are permeable to fluid.[15] Moreover, single-bottle self-etch adhesives without a comparatively more hydrophobic bonding resin layer are permeable to fluid after polymerization.

Likewise, the worse sealing ability of water-based single-bottle adhesives (Prompt in this study) is consistent with a previous report.[18] Conversely, an acetone-based single-bottle self-etch adhesive (SB M) revealed far better sealing ability in this study. However, Tay and others[6] demonstrated that the single-bottle self-etch adhesive served as a permeable membrane, irrespective of which solvents were used (water-, ethanol- or acetone-based adhesives). The findings of the current study indicated that the reduction of dentinal permeability was associated with the individual dentin adhesives used, not with the categories/generations of the dentin adhesives. Water droplets trapped in the dentin-bonding layer will be present as water-in-oil or oil-in-water emulsion at the tooth hard tissue-adhesive interfaces.[19] After the volatile solvents evaporate, concentration of the nonvolatile solvents increases. Residual water in water-, acetone- or alcohol-based primers cannot be completely evaporated on dentin surfaces due to the high surface tension of water,[20] water transudation from the dentin[7] and hydrogel formation of the HEMA present in the adhesive systems.[17] Residual water might hinder complete co-polymerization of the hydrophobic bonding resin and the hydrophilic primer.[17] Inter-connecting channels (water trees) have been shown to exist within the bonding layers,[5-6,16,21-22] allowing fluid flow through the bonding layer into the dentin tubules. This might be the reason for the observation that dentin adhesives can reduce dentin permeability but cannot completely block fluid/ion diffusion through dentin.

Although the non-polymerizable Gluma desensitizer revealed the worse sealing ability of the dentinal tubules in this study, it still possessed a certain ability to reduce dentinal permeability compared with the control group ($p<0.05$). GD achieves its effects by the precipitation of plasma protein caused by glutaraldehyde. Protein precipitation reduces dentinal permeability and occludes the peripheral dentinal tubules. The intrinsic septa that results from precipitation of the plasma protein could not mechanically block dentin permeability in this study; however, the desensitizer is capable of reducing intratubular serum albumin flow onto exposed dentin surfaces.[23] Furthermore, the desensitizer that was covered with a comparatively more hydrophobic bonding resin layer revealed a better sealing ability than the desensitizer itself.

According to the current study, the dentin adhesives/desensitizer could not completely block dentin permeability; however, dentin adhesives/desensitizer are successfully used in clinical practice to relieve dentin hypersensitivity. This can be explained by the fact that topical application of dentin adhesives/desensitizer over dentin surfaces can block comparatively quicker fluid percolation through the dentinal tubules but cannot stop slow water/ion diffusion into and out of the dentinal tubules. This comparatively quicker fluid percolation through the dentinal tubule contributes to dentin hypersensitivity.

The hydraulic conductance of human dentin is widely used to test dentin permeability in order to screen out the potential for dentin adhesives/ desensitizer used for desensitizing purposes.[24-25] Pulpal pressure and outward flow of dentin tubular fluid can reduce dye penetration into dentin, but it cannot stop dye penetration into dentin. Therefore, the dye penetration method without pulpal pressure used in this study is a good alternative to test the sealing ability of dental adhesives/desensitizer.[26]

Interestingly, the micro-morphological findings in this study are in accordance with the degree of dye penetration. In cases of experimental groups characterized by low dye penetration scores, the corresponding SEM micrographs indicated well-sealed dentinal surfaces. In cases of more extensive dye penetration, poorly sealed dentinal surfaces were observed in corresponding SEM pictures. The micro-morphologic findings in the current study revealed that the sufficient micro-emulsion co-polymerization of the hydrophobic bonding resin upon the layer of hydrophilic primers was important for sealing dentinal tubules. Although

Table 2: *Sealing Ability of the Dentin Adhesives/Desensitizer Used in Groups I-XI and Results of Statistical Analysis (Kruskal-Wallis H Test)*

Codes of Groups	**Frequency Distribution of Dye Penetration Scores**					**H-test**	
	0	**1**	**2**	**3**	**4**	**Mean Rank**	***p*-values**
Ctr	0	0	0	0	10	102.50	0.000
GD	0	0	1	4	5	91.60	
GDCB	0	1	4	5	0	71.45	
APMB	0	6	2	2	0	51.00	
CLB A	3	5	2	0	0	33.45	
CLB AB	6	3	1	0	0	23.00	
SBMP	0	4	2	4	0	60.90	
Ecusit	2	5	2	1	0	40.95	
Prompt	0	1	5	3	1	71.05	
SB M	8	1	1	0	0	17.90	
SB exp	0	6	4	0	0	46.70	

χ^2 = 75.419, n=10

Table 3: *Pairwise Comparison of Dye Penetration Scores in Groups I to XI (p-values of Nemenyi test)*

Codes	**Ctr**	**GD**	**GDCB**	**APMB**	**CLB A**	**CLB AB**	**SBMP**	**Ecusit**	**Prompt**	**SB M**
SB exp	<0.01	<0.01	<0.01	n.s.	<0.01	<0.01	<0.01	n.s.	<0.01	<0.01
SB M	<0.01	<0.01	<0.01	<0.01	<0.01	n.s.	<0.01	<0.01	<0.01	
Prompt	<0.01	<0.01	n.s.	<0.01	<0.01	<0.01	<0.05	<0.01		
Ecusit	<0.01	<0.01	<0.01	<0.05	n.s.	<0.01	<0.01			
SBMP	<0.01	<0.01	<0.05	<0.05	<0.01	<0.01				
CLB AB	<0.01	<0.01	<0.01	<0.01	<0.05					
CLB A	<0.01	<0.01	<0.01	<0.01						
APMB	<0.01	<0.01	<0.01							
GDCB	<0.01	<0.01								
GD	<0.05									

p*<0.05 was accepted as statistically significant. n.s.: no significance.*

the dentin adhesives could not completely block dentin permeability, they could effectively occlude the patent dentinal tubules and significantly reduce dentin permeability. These findings indicate that most of the current dentin adhesives possess an ability to reduce dentin permeability to some degree, especially, adhesives such as SB M, CLB AB, Ecusit and SB exp.

The hypothesis in this study is fully accepted. Topical application of dentin adhesives on the dentinal surface can significantly reduce dentin permeability, but it cannot completely stop fluid diffusion into the dentin tubules.

CONCLUSIONS

None of the dentin adhesives/desensitizer that was tested could completely stop fluid diffusion through the dentinal tubules; however, most of the dentin adhesives could effectively occlude the exposed dentin tubules and significantly reduce dentin permeability.

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