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Dentin Permeability and Nanoleakage of Universal Adhesives in Etch-and-rinse vs Self-etch Modes

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Clinical Relevance

Universal adhesives do not seem to have the ability to effectively seal dentin, regardless of the mode of application.

SUMMARY

Purpose: This study evaluates the reduction in dentin permeability (P) and the expression of nanoleakage in resin-dentin interfaces made with universal adhesives, comparing the self-etch mode of application with the etch-and-rinse mode of application.

Methods and Materials: To measure dentin P at the baseline and after adhesive polymerization, 80 extracted noncarious human third molars (N=80) were sectioned to obtain 0.7-mm-thick midcoronal dentin disks. The specimens were randomly assigned to 8 groups according to the different adhesive systems and application modes used: Scotchbond Universal (SBU; etch-and-rinse [ER] mode vs self-etch [SE] mode), OptiBond XTR (OPT; etch-and-rinse mode vs self-etch mode), Clearfil Universal Bond Quick (CL; etch-and-rinse mode vs self-etch mode), and Adhese Universal

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(ADH; etch-and-rinse mode vs self-etch mode). A two-way analysis of variance with interaction was used to evaluate the P reduction differences among the groups (α=0.05). Sixteen additional 0.7-mm-thick dentin disks were similarly prepared and treated, immersed in 50 wt% ammoniacal silver nitrate, and then processed according to conventional methods for analysis under transmission electron microscopy.

Results: The differences in P reduction among the adhesives were significant (*p*=0.029). For OPT and ADH (*p*=0.0304 and *p*=0.0120, respectively), the P reduction was higher in the ER mode than in the SE mode. For CL and SBU, no differences in P reduction was observed between the two application modes (*p*>0.05). All the groups showed some pattern of nanoleakage.

Conclusions: No adhesive system completely reduced dentin P. Dentin sealing was higher for OPT and ADH in the ER mode, while for SBU and CL, there was no difference between the two modes. All the adhesive systems showed nanoleakage.

INTRODUCTION

Despite the advancements in dentinal adhesive systems, there are still some cases where resin composite restorations are associated with postoperative sensitivity. The behavior of adhesive systems depends on the quality of their components (chemical composition) and their properties.[1,2] Their formulations should be based on hydrophobic and high molecular weight monomers, without additives such as water and solvents.[3] However, due to the moist nature of dentin, to have good wetting and penetration between the collagen fibers in the demineralized dentin, hydrophilic components and solvents must be incorporated into the composition of these materials.[1]

A new version of adhesive systems known as universal or multimode adhesives,[4,5] consists of mixtures of acidic, hydrophilic, and hydrophobic monomers; organic solvents; additives; and, necessarily, water in their composition. Thus, as with most other types of adhesive systems,[6,7] the hydrophilic property of the polymers formed by these universal adhesive systems allows water to diffuse from the dentin or the external medium through the hybrid layer. Consequently, dilution of the primer components may occur, impairing the infiltration of resin monomers into the demineralized dentin. If there is inadequate penetration and hybridization of the resin monomers demineralized dentin zone and inside the tubules—forming a continuous hybrid layer and sealing the dentinal tubules—then several outcomes are possible: dentin fluid movement and nanoleakage,[8-12] hydrolyses of the collagen and degradation of the resins,[13-18] leaching of the resin components,[9] and, ultimately, an increased risk of dentin P[19] and postoperative sensitivity.[20-22]

Pashley and others[23] referred to two different types of dentin P: intradentinal P and transdentinal P. Intradentinal P is responsible for the monomer diffusion from etched tubule lumens into the surrounding collagen to hybridize the resin tags to the walls of the tubules or for the diffusion of adhesive monomers into the dentin matrix between the dentin tubules.[24] Transdentinal P, also called hydrodynamic fluid flux, is the movement of fluid across dentin within the dentin tubules.[25] According to the hydrodynamic theory of dentin sensitivity, transdentinal P is responsible for dentin sensitivity of both exposed dentin and restored dentin (postoperative sensitivity). Considering that universal adhesive systems have different application modes, ie, etch-and-rinse (ER) and self-etch (SE), it is necessary to clarify whether the universal adhesives have the same capacity to seal the dentin as the adhesives they replace to avoid cases of postoperative sensitivity.

The P characteristics of the hybrid layer or resin-dentin interdiffusion zone can be studied separately using either quantitative or qualitative methods. Quantitatively, the dentin P measurement method described by Outhwaite and others,[26] and Pashley,[27] can be used, and qualitatively, transmission electron microscopy can be employed to measure the ultramorphology and nanoleakage. In this study, both methods were used, not only to have a quantitative estimation of the dentin P reduction achieved but also to assess the exact location in the dentin-adhesive interface from where the P is coming.

Therefore, the purpose of this study was to evaluate the reduction in dentin P and nanoleakage of a resin-dentin interface made with universal adhesives, using the SE technique and the ER technique. The null hypothesis was as follows: no differences in dentin P and nanoleakage will be observed among the 8 experimental groups.

METHODS AND MATERIALS

Specimen Preparation for Permeability Study

Eighty recently extracted human third molars that were intact and without evidence of caries or restorations were used in this study. Prior to preparation, the teeth were randomly selected from a group of teeth, stored in 0.5% chloramine-T (Sigma-Aldrich Chemical Co, St

Louis, MO, USA) at 4°C for one week, and then left in distilled water at 4°C, according to the ISO TR 11405 Standard developed by the International Organization for Standardization.

The teeth were sectioned parallel to the occlusal surface 2 mm below the cementoenamel junction to remove the roots and occlusally to obtain a crown segment containing at least 0.7-mm deep dentin, using a low-speed diamond saw (IsoMet; Buehler, Lake Bluff, IL, USA) with water coolant. The specimen thickness was measured with a digital micrometer (Micro 2000, 0-25 mm; Moore & Wright, Sheffield, UK) to obtain the exact thickness.

The pulpal surfaces were prepared with a diamond bur to gently remove the pulp tissue, which created a smear layer. Then the pulpal surfaces of the specimens were conditioned with 37% phosphoric acid gel (Total Etch; Ivoclar Vivadent, Schaan, Liechtenstein) for 1 minute to completely remove the smear layer and smear plugs, opening all the tubules and thus allowing the fluid to freely flow within the dentin tubules during permeability (P) measurements. The specimens were then glued on standard acrylic pieces (1 cm × 0.5 cm × 1 cm) with an impression compound. These acrylic pieces have a central channel that allows the passage of an 18-gauge needle connected to a hydraulic system. The dentin surface was ground with a 600 grit SiC paper (Carbimet Grit 600/P1200; Buehler) for 60 seconds under water irrigation to produce a uniform smear layer, in accordance with the ISO TR 11405 Standard.

The specimens were treated in random order to avoid any bias due to a particular sequencing of treatments. Thus, the 80 specimens were randomly assigned to 8 groups, with 10 specimens per group: (1) Scotchbond Universal (SBU; 3M Oral Care, Seefeld, Germany) in the ER mode, (2) OptiBond XTR (OPT; Kerr Corporation, Orange, CA, USA) in the ER mode, (3) Clearfil (CL) Universal Bond Quick (Kuraray, Okayama, Japan) in the ER mode, (4) Adhese Universal (ADH; Ivoclar Vivadent, Schaan, Liechtenstein) in the ER mode, (5) SBU (3M Oral Care) in the SE mode, (6) OPT (Kerr Corporation) in the SE mode, (7) CL (Kuraray) in the SE mode, and (8) ADH (Ivoclar Vivadent) in the SE mode. The adhesives were applied according to the detailed instructions provided by the manufacturers. The composition of the adhesives, original manufacturer instructions, and detailed manufacturer instructions are described in Table 1. Dentin P was measured for each specimen at three different points of time:(1) before etching the occlusal side; (2) after etching, which served as the baseline (P_B); and (3) at the end of adhesive polymerization (P_A).

Dentin Permeability Measurements

Each specimen was connected to a hydraulic pressure system, as shown in Figure 1, with 37 cmH_2O, which is close to normal pulpal pressure.[28-30] The fluid flow was measured by following the movement of an air bubble trapped within a glass capillary tube (0.7 mm inside diameter, Microcaps; Fisher Scientific, Atlanta, GA, USA) that was positioned between the pressure reservoir and the crown segment, according to the hydraulic conductance protocol reported by Pashley and Depew.[31]

The absence of fluid conductance before the exposure of the occlusal dentin was confirmed by separately attaching 5 intact crown segments to the testing apparatus (as described above) and observing the (absence of) fluid movement for 2 hours.[28,29]

During the application of the adhesive system, the pressure was interrupted to avoid any interference with the effectiveness of the adhesive system.[30,32] The progression of the air bubble was measured every 2 minutes over a 6-minute interval to determine the rate of saline solution flow in millimeters per minute.

Calculations to Determine Dentin Permeability

The dentin P of each specimen was measured at 2 points in time: (1) after conditioning with the acid (P_B measurement), and (2) P_A. These 2 measurements were used to calculate, as a ratio, the reduction in dentin P. The value of dentin P, measured after conditioning with the acid (P_B), was initially assigned as 100%. The P_A of each specimen was determined after the application of the adhesive system, and the adhesive system was applied without taking the specimen out of the system. The reduction in dentin P after P_A was expressed as a percentage of this maximum value [$100 - (P_A/P_B \times 100)$]. Thus, each specimen served as its own control.

Statistical Analysis

Sample size calculations were performed using the G*Power Program Statistical Analysis,[33,34] with α=0.05, a desired power of 80%, and data from the pilot study. The results were statistically analyzed by two-way analysis of variance (ANOVA) with interaction (IBM SPSS Statistics version 23.0; IBM, Armonk, NY, USA) to evaluate the effects of the mode of application and the choice of adhesive on the reduction in dentin P.

Specimen Preparation for Nanoleakage Study

Sixteen additional 0.7-mm-thick dentin disks were prepared similar to those in the P study. Two dentin disks were used for each of the 8 adhesive groups, and the adhesives were applied in a manner similar to that in the P study. After storing them in distilled water at

Table 1: *Components, Compositions (Information Supplied by the Manufacturer) and Application Mode of the Tested Adhesives*

Material	**pH**	**Components**	**Manufacturer's Instructions Detailed**
Scotchbond Universal (3M Oral Care)	2.7	Bis-GMA; 10-MDP; hydroxyethyl methacrylate; decamethylene dimethacrylate; ethanol; water; silane-treated silica; 2-propenoic acid, 2-methyl-, reaction products with 1,10-decanediol and phosphorous oxide (P_2O_5); copolymer of acrylic and itaconic acid; dimethylaminobenzoat; camphorquinone; (dimethylamino)ethyl methacrylate; methyl ethyl ketone	Etch-and-rinse mode: 1. Apply phosphoric acid etching gel (Total Etch; Ivoclar Vivadent) for 15 seconds. Rinse the occlusal surface with water. Remove the excess water using a moist cotton pellet, so that the surface remains shiny and visibly moist. 2. Apply adhesive, using a microbrush for 20 seconds. 3. Dry the surface, beginning with a soft blow of air from a distance of approximately 10 cm (the air pressure was increased while decreasing distance), finishing at a distance of approximately 1–2 mm from the surface at maximum air pressure. 4. Light-cure for 10 seconds. Self-etch mode: 1. Apply adhesive, using a microbrush for 20 seconds. 2. Dry the surface, beginning with a soft blow of air from a distance of approximately 10 cm (the air pressure was increased while decreasing distance), finishing at a distance of approximately 1-2 mm from the surface at maximum air pressure. 3. Light-cure for 10 seconds.
Clearfil Universal Bond Quick (Kuraray)	2.3	10-MDP; Bis-GMA; HEMA; hydrophilic amide monomers; colloidal silica, silane coupling agent; sodium fluoride; dl-camphorquinone; ethanol; water	Etch-and-rinse mode: 1. Apply phosphoric acid etching gel (Total Etch; Ivoclar Vivadent) for 15 seconds. Rinse the occlusal surface with water. Remove the excess water using a moist cotton pellet, so that the surface remains shiny and visibly moist. 2. Apply adhesive, using a microbrush for 20 seconds. 3. Dry the surface, beginning with a soft blow of air from a distance of approximately 10 cm (the air pressure was increased while decreasing distance), finishing at a distance of approximately 1- mm from the surface at maximum air pressure. 4. Light-cure for 10 seconds. Self-etch mode: 1. Apply adhesive, using a microbrush for 20 seconds. 2. Dry the surface, beginning with a soft blow of air from a distance of approximately 10 cm (the air pressure was increased while decreasing distance), finishing at a distance of approximately 1-2 mm from the surface at maximum air pressure. 3. Light-cure for 10 seconds.

Table 1: *Continued*			
Adhese Universal (Ivoclar Vivadent)	2.5	10-MDP; dimethacrylate resins; HEMA; ethanol; water; MCAP; fillers; initiators	Etch-and-rinse mode: 1. Apply phosphoric acid etching gel (Total Etch, Ivoclar Vivadent) for 15 seconds. Rinse the occlusal surface with water. Remove the excess water using a moist cotton pellet, so that the surface remained shiny and visibly moist. 2. Apply adhesive, using a microbrush for 20 seconds. 3. Dry the surface, beginning with a soft blow of air from a distance of approximately 10 cm (the air pressure was increased while decreasing distance), finishing at a distance of approximately 1–2 mm from the surface at maximum air pressure. 4. Light-cure for 10 seconds. Self-etch mode: 1. Apply adhesive, using a microbrush for 20 seconds. 2. Dry the surface, beginning with a soft blow of air from a distance of approximately 10 cm (the air pressure was increased while decreasing distance), finishing at a distance of approximately 1-2 mm from the surface at maximum air pressure. 3. Light-cure for 10 seconds.
Optibond XTR (Kerr Corporation)	3.4	Primer: GPDM, various methacrylate monomers; HEMA; acetone; ethanol; water; CQ initiator Adhesive: GPDM; tri-functional monomer; HEMA; ethanol; disodium hexafluorosilicate; MEHQ	Etch-and-rinse mode: 1. Apply phosphoric acid etching gel (Total Etch, Ivoclar Vivadent) for 15 seconds. Rinse the occlusal surface with water. Remove the excess water using a moist cotton pellet, so that the surface remains shiny and visibly moist. 2. Apply the primer, using a microbrush for 20 seconds. 3. Dry the surface, beginning with a soft blow of air from a distance of approximately 10 cm (the air pressure was increased while decreasing distance), finishing at a distance of approximately 1-2 mm from the surface at maximum air pressure. 4. Apply adhesive, using a microbrush for 20 seconds. 5. Light-cure for 10 seconds. Self-etch mode: 1. Apply the primer, using a microbrush for 20 seconds. 2. Dry the surface, beginning with a soft blow of air from a distance of approximately 10 cm (the air pressure was increased while decreasing distance), finishing at a distance of approximately 1–2 mm from the surface at maximum air pressure. 3. Apply adhesive, using a microbrush for 20 seconds. 4. Dry the surface, beginning with a soft blow of air from a distance of approximately 10 cm (the air pressure was increased while decreasing distance), finishing at a distance of approximately 1-2 mm from the surface at maximum air pressure. 5. Light-cure for 10 seconds.

Abbreviations: 10-MDP, 10-Methacryloyloxydecyl dihydrogen phosphate; Bis-GMA, bisphenol A-glycidyl methacrylate; GPDM, glycerol phosphate dimethacrylate; HEMA, 2-hydroxyethylmethacrylate; MCAP, methacrylated carboxylic acid polymer; MEHQ, methoxyphenol; pH, potential hydrogen.

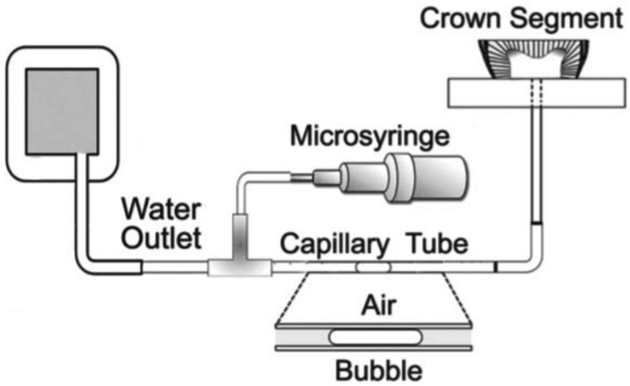

Figure 1. *Schematic diagram of the apparatus used for dentin permeability measurements (adapted from Sahin & others[18]).*

37°C for 24 hours, a 1-mm-wide slab containing the resin-dentin interface was prepared from the widest portion of each bonded disk. They were immersed in a 50 wt% ammoniacal silver nitrate tracer solution (Sigma Aldrich, St. Louis, MO, USA) for 24 hours according to the silver impregnation protocol for nanoleakage examination reported by Tay and Pashley.[16] After the reduction of the diamine silver ions, the silver-impregnated slabs were processed for transmission electron microscope (TEM) examination without demineralization. Epoxy resin-embedded sections 90-nm-thick were prepared and examined unstained[16] using a transmission TEM (FEI Tecnai G2 Spirit BioTWIN, operating at 120 keV, equipped with an Olympus-SIS Veleta CCD camera).

RESULTS

Dentin Permeability Study

The Kolmogorov-Smirnov test of normality at $\alpha=0.05$ revealed that the data presented a normal distribution, and Levene's test was used to verify the homogeneity of the variances ($p=0.5596$), enabling a parametric analysis.

The data on percent P reduction are summarized in Table 2 by adhesive types and application modes as a mean and standard deviation; the median, range (minimum, maximum), and p-values from the normality test are also presented. The medians and means were similar and consistent for each group, and the normality tests were not significant ($p>0.10$) for any of the groups. OPT in the ER mode had the largest mean (83.8%), and CL in the ER mode had the lowest mean (35.8%). Tables 3 and 4 present the results from the two-way ANOVA with interaction. Table 3 lists the estimates, the standard error, and the p-values.

The significant interaction suggested that the effect of adhesive types on P reduction depended on the mode or that the mode effect varied with the adhesive types. Table 3 presents the results in detail. In the ER mode, the adhesive type was significant ($p=0.0007$), while in the SE mode it was less significant ($p=0.0170$). In the ER mode, the results obtained for OPT were significantly greater than those obtained for SBU and CL ($p<0.05$), and the results obtained for ADH were significantly better than those obtained for CL. In the SE mode, only a marginal significance was found for OPT over ADH ($p=0.0577$), with OPT obtaining a better result. For OPT and ADH ($p=0.0304$ and $p=0.0120$, respectively), the P reduction was higher in the ER mode than in the SE mode. For CL and SBU, no differences in P reduction were observed between the 2 application modes ($p>0.05$).

Nanoleakage Study

The OPT ER group did not show significant nanoleakage, and isolated spots of silver grains (Figure 2, black arrows) could only be discerned at high magnification. They were randomly distributed

Table 2: *Data Description of Percentage Permeability Reduction (%)*

Mode and Adhesive	Mean ± SD (%)	Median (min, max)
Etch-and-rinse		
Scotchbond Universal	53.2 (37.6)	54.8 (0.0, 100.0)
Optibond XTR	83.8 (15.1)	89.2 (50.0, 100.0)
Clearfil Universal Bond Quick	35.8 (29.6)	29.2 (0.0, 85.7)
Adhese Universal	66.0 (19.3)	66.7 (37.5, 100.0)
Self-Etch		
Scotchbond Universal	54.5 (19.8)	50.0 (25.0, 95.2)
Optibond XTR	58.6 (22.1)	60.9 (25.0, 100.0)
Clearfil Universal Bond Quick	56.4 (25.2)	56.3 (20.0, 95.0)
Adhese Universal	36.6 (28.4)	35.9 (0.0, 86.4)

Table 3: *Results from Two-way ANOVA with Interaction*

Study Groups		**Estimates**	**Standard Error**	***p*-value**	***p*-value (overall)**
Adhesive	Scotchbond Universal	17.9	11.4	0.1207	0.0170
	Optibond XTR	22.2	11.4	0.0577	
	Clearfil Universal Bond Quick	19.9	11.4	0.0859	
	Adhese Universal	0.00	—	—	
Mode	Etch-and-rinse	29.4	11.4	0.0120	—
	Self-etch	0.00	—	—	
Adhesive*mode	ER*SBU	-30.7	16.1	0.0613	0.0081
	ER*OPT	-4.2	16.1	0.7946	
	ER*CL	-50.0	16.1	0.0028	
	ER*ADH	0.00	—	—	

Abbreviations: ADH, Adhese Universal; ANOVA, analysis of variance; CL, Clearfil Universal Bond Quick; ER, etch-and-rinse; OPT, OptiBond XTR; SBU, Scotchbond Universal; SE, self-etch.

exclusively in the hybrid layer (Figure 2). In this group, the hybrid layer was approximately 2-μm thick.

In the OPT SE group, 2 types of nanoleakage patterns could be observed within the resin-dentin interfaces: a spotted pattern (Figure 3, black arrows) and a reticular pattern (Figure 3, gray arrows). The spotted pattern consisted of isolated spots of silver grains that were observed in the hybrid layer in various amounts. The reticular pattern consisted of discontinuous islands of silver deposits exclusively observed in the hybrid layers

Table 4: *Stratified Evaluation of the Effects of Adhesives and Mode on Permeability Reduction*

Adhesive Effect	**Estimate**	**Standard Error**	***p*-value**
Given mode: ER			$p<0.0001$ (overall)
SBU vs OPT	-30.6	11.4	0.0092
SBU vs CL	17.4	11.4	0.1321
SBU vs ADH	-12.8	11.4	0.2674
OPT vs CL	48.0	11.4	<.0001
OPT vs ADH	17.8	11.4	0.1234
CL vs ADH	-30.2	11.4	0.0101
Given mode: SE			$p=0.0170$ (overall)
SBU vs OPT	-4.1	11.4	0.7212
SBU vs CL	-2.0	11.4	0.8649
SBU vs ADH	17.9	11.4	0.1207
OPT vs CL	2.1	11.4	0.8518
OPT vs ADH	22.0	11.4	0.0577
CL vs ADH	19.9	11.4	0.0859
Mode effect			
Given Adhesive = SBU	-1.3	11.4	0.9131
Given Adhesive = OPT	25.2	11.4	0.0304
Given Adhesive = CL	-20.6	11.4	0.0755
Given Adhesive = ADH	29.4	11.4	0.0120

Abbreviations: ADH, Adhese Universal; CL, Clearfil Universal Bond Quick; ER, etch-and-rinse; OPT, OptiBond XTR; SBU, Scotchbond Universal; SE, self-etch.

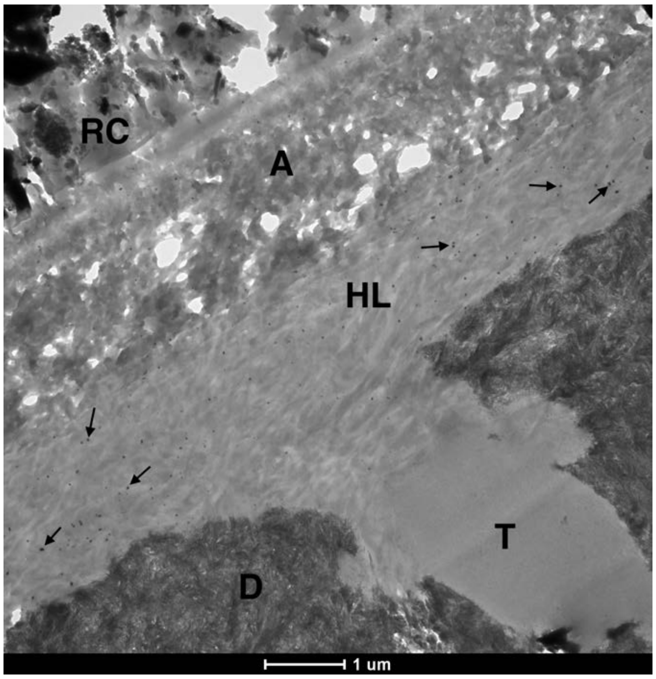

Figure 2. *Transmission electron microscope (11,500x) micrograph of representative area of nonstained, demineralized, nonsilver-impregnated sections of the OPT ER group. Abbreviations: A, adhesive layer; D, mineralized dentin; ER, etch-and-rinse; HL, hybrid layer; OPT, OptiBond XTR; RC, resin composite; T, dentin tubule.*

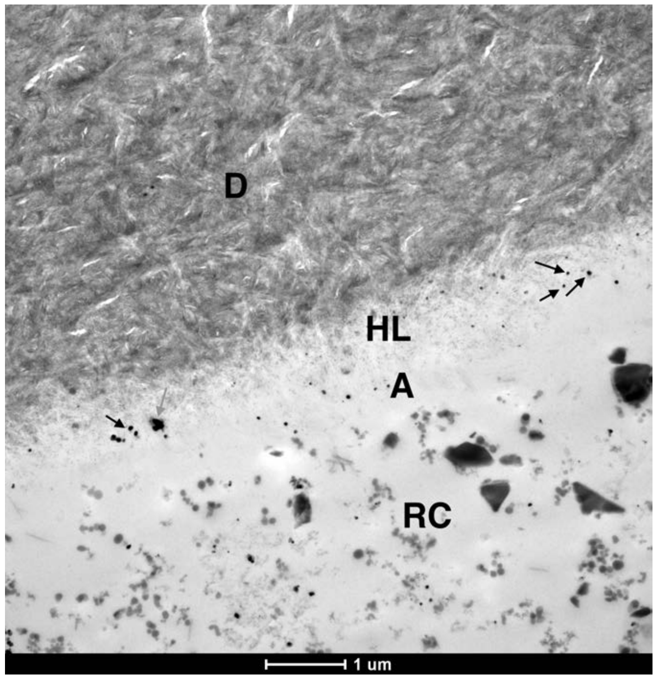

Figure 3. *Transmission electron microscope (11,500x) micrograph of representative area of nonstained, demineralized, nonsilver-impregnated sections of the OPT SE group. A, adhesive layer; D, mineralized dentin; ER, etch-and-rinse; HL, hybrid layer; OPT, OptiBond XTR; RC, resin composite; SE, self-etch.*

or in the hybridized areas of resin tags. In this group, a thin hybrid layer was observed (<0.5 μm).

The SBU ER group did not show any significant nanoleakage. Isolated spots of silver grains (Figure 4, black arrows) that were distributed in the hybrid layer were noted, with a higher concentration at the base of the hybrid layer. A hybrid layer with a thickness between 0.5 and 1 μm was observed, with good infiltration throughout.

In the SBU SE group, isolated spots of silver grain (Figure 5, black arrows) were randomly distributed across a thin hybrid layer (about 0.5-μm thick).

In the CL ER group, a hybrid layer with a thickness of approximately 2.5 μm was observed. The hybrid layer was impregnated with an extensive spotted type (Figure 6, black arrows) of nanoleakage and reticular silver deposits (Figure 6, gray arrows).

In the CL SE group, a thin hybrid layer was observed (<0.5 μm), with the presence of a linearly distributed reticular nanoleakage pattern along the base. A spotted type of nanoleakage (Figure 7, black arrows) was also noted.

In the ADH ER group, a hybrid layer of about 2-μm thick was observed, with the presence of 2 nanoinfiltration patterns: a spotted-type pattern (Figure 8, black arrows) and reticular silver deposits (Figure 8, gray arrows). A layer of dendritic silver-impregnated water channels (ie, water trees shown as white arrows) can also be seen in the hybrid layer.

In the ADH SE group, a thin hybrid layer (<0.5 μm) was observed, with a spotted nanoleakage pattern (Figure 9, black arrows) randomly distributed. Occasionally, at the base of the hybrid layer, silver deposits were also agglomerated in a reticular pattern (Figure 9, gray arrows).

DISCUSSION

Studies of P characteristics of dentin and their interactions with dentin adhesive systems may help to explain some of the reasons for restorative failure or postoperative dentin sensitivity. Prati and Pashley[35] found a significant correlation between bond strength and dentin P of the restoration for some adhesive systems. Moreover, in some studies, the application of the restorative material was not able to achieve a perfect seal and block the passage of fluid.[36,37]

A hydraulic conductance study has advantages over other types of leakage studies[27] because it gives a quantitative measurement of the interfacial leakage, assesses whether the dentin tubules are effectively sealed, allows repeated measurements on the same specimen longitudinally and nondestructively, and,

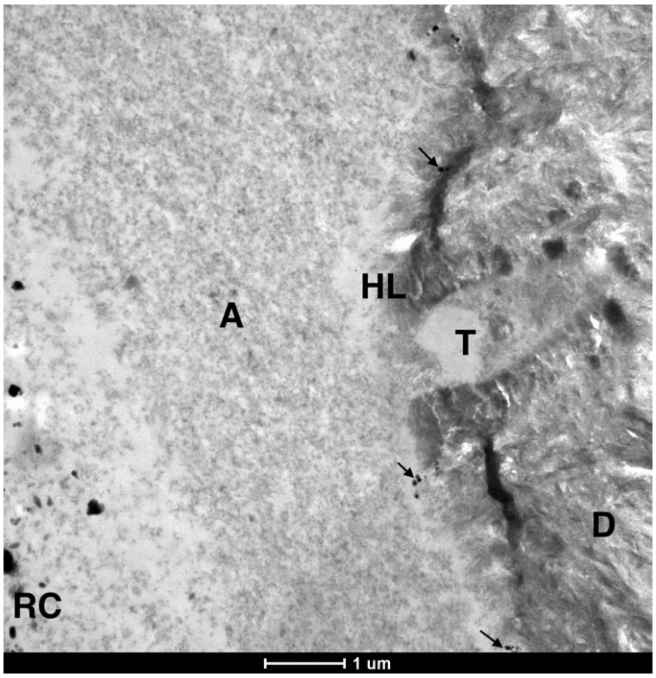

Figure 4. *Transmission electron microscope (11,500x) micrograph of representative area of nonstained, demineralized, nonsilver-impregnated sections of the SBU ER group. Abbreviations: A, adhesive layer; D, mineralized dentin; ER, etch-and-rinse; HL, hybrid layer; RC, resin composite; SBU, Scotchbond Universal; T, dentin tubule.*

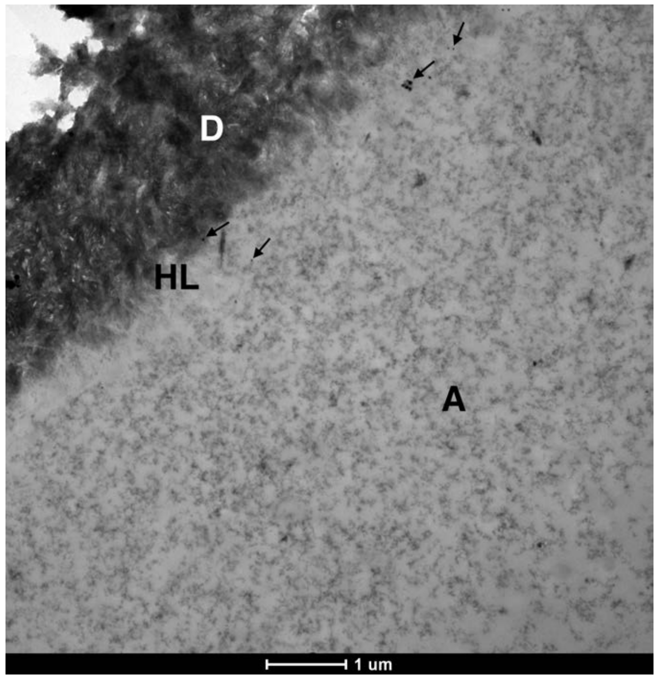

Figure 5. Transmission electron microscope (11,500x) micrograph of representative area of nonstained, demineralized, nonsilver-impregnated sections of the SBU SE group. Abbreviations: A, adhesive layer; D, mineralized dentin; HL, hybrid layer; SBU, Scotchbond Universal; SE, self-etch.

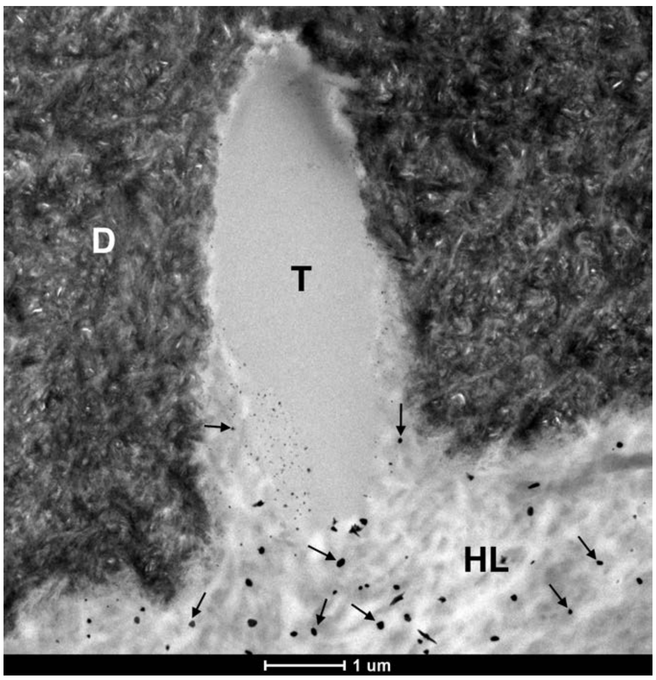

Figure 6. *Transmission electron microscope (11,500x) micrograph of representative area of nonstained, demineralized, nonsilver-impregnated sections of the CL ER group. Abbreviations: A, adhesive layer; CL, Clearfil Universal Bond Quick; D, mineralized dentin; ER, etch-and-rinse; HL, hybrid layer; T, dentin tubule.*

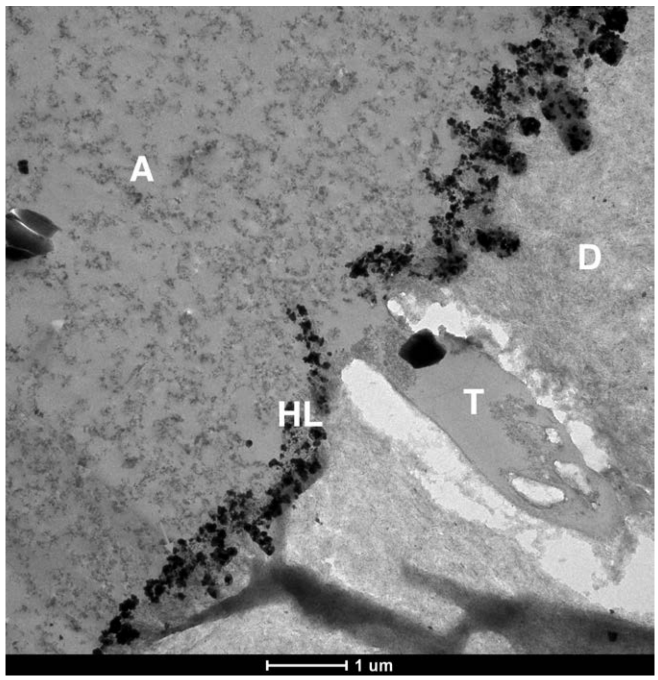

Figure 7. *Transmission electron microscope (11,500x) micrograph of representative area of nonstained, demineralized, nonsilver-impregnated sections of the CL SE group. Abbreviations: A, adhesive layer; CL, Clearfil Universal Bond Quick; D, mineralized dentin; HL, hybrid layer; RC, resin composite; SE, self-etch; T, dentin tubule.*

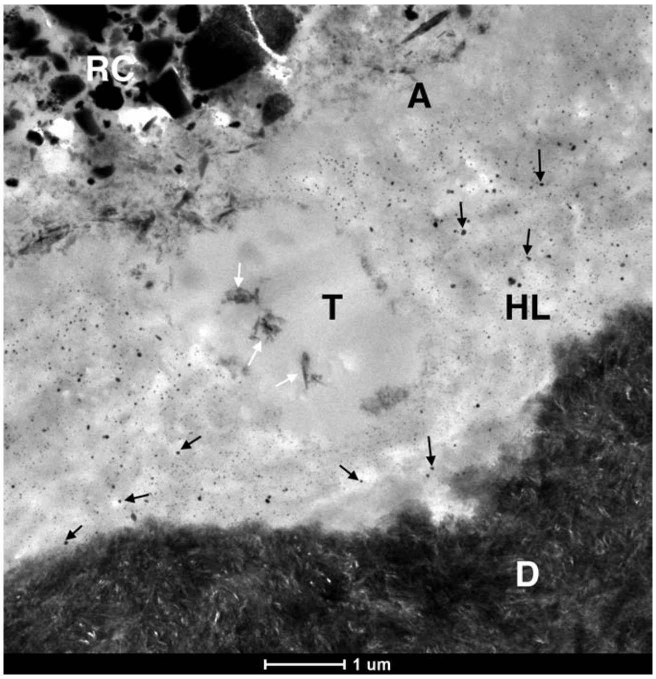

Figure 8. *Transmission electron microscope (11,500x) micrograph of representative area of nonstained, demineralized, nonsilver-impregnated sections of the ADH ER group. Abbreviations: A, adhesive layer; ADH, Adhese Universal; D, mineralized dentin; ER, etch-and-rinse; HL, hybrid layer; RC, resin composite; T, dentin tubule.*

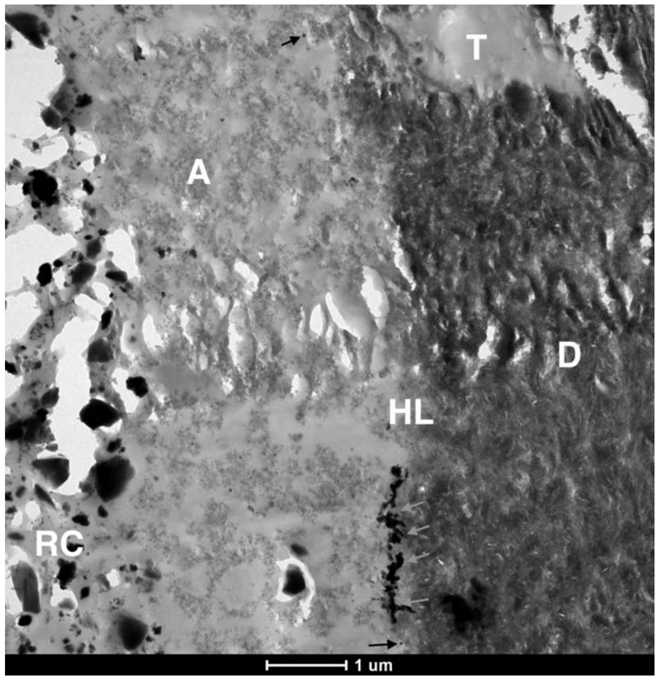

Figure 9. *Transmission electron microscope (11,500x) micrograph of representative area of nonstained, demineralized, nonsilver-impregnated sections of the ADH SE group. Abbreviations: A, adhesive layer; ADH, Adhese Universal; D, mineralized dentin; HL, hybrid layer; RC, resin composite; SE, self-etch; T, dentin tubule.*

finally, measures dentin P_B (after etching) and P_A (after the adhesive polymerization), allowing each specimen to serve as its own control.

Quantitatively measuring dentin P by means of hydraulic conductance[26] involves forcing a fluid through the dentin by applying pressure. In this study, high pressure could have flushed out the resin tags, resulting in an overestimation of dentin P. To avoid any disturbance of the intratubular content—the hybrid layer or the resin tags—a low hydraulic pressure of 37 cmH_2O was used in this study. The use of this low hydraulic pressure, which is close to the physiological pressure (14 cmH_2O),[38] is expected to result in hydraulic conductance measurements comparable to reality.

According to Tao and Pashley,[19] it is desirable that all adhesive systems reduce dentin P or, at least, not increase it.

All the adhesive systems used in this study were able to reduce P (35.8%-83.8%); however, no adhesive system was able to completely seal the dentin after Pa, which is in agreement with Hashimoto,[39] who stated that all adhesive systems are permeable to water but at different levels. The most satisfactory results for reduction in dentin P were obtained with OPT ER, which may indicate that the separate application of primer and adhesive is more favorable to achieve good hybridization.[40] Therefore, hybridization effectiveness can be reduced by simplifying the application procedure of adhesive systems through combining the primer and adhesive into a single application step.

OPT does not contain the 10-methacryloyloxy-decyl-dihydrogen-phosphate (10-MDP) monomer, so these results differ from the results of Munoz and others,[41] in which only the adhesive systems containing 10-MDP were able to reduce nanoleakage.

When applying phosphoric acid, which is necessary for the ER technique,[42] the smear layer and the smear plugs are removed, increasing dentin P 4-9 times depending on the diameter of the tubules and their density at the site of evaluation.[43] Considering that no smear layer removal is done in the SE technique, it could be assumed that the use of this technique would be advantageous to minimize dentin P.[42,44]

However, this was not the case in this study because no statistically significant differences were found between the application modes in the CL and SBU adhesive systems, but differences were observed for the OPT and ADH adhesive systems (p=0.0304 and p=0.0120, respectively), and the maximum P reduction was obtained by the ER technique. One possible explanation for this may be the poor polymer formation that constitutes the hybrid layer produced by these simplified adhesive systems in the SE mode. Nevertheless, in these cases, changes in the hybrid layer formation would not be attributed to excess moisture due to the removal of the smear layer and the smear plugs, but rather to the retention of solvents and water that make up the formulation of these adhesive systems.[45]

An examination of the results demonstrates that the adhesive system for any of the groups were not capable of completely reducing the dentin P produced after etching. This seems to indicate that the use of a universal adhesive may present clinically postoperative sensitivity, regardless of the mode of application: SE or ER.[46]

All the adhesive systems in this work presented at least one nanoleakage pattern, irrespective of the application technique used. The nanoleakage results are consistent with the results of the dentin P study and with other studies,[7,47] where no adhesive has been fully able to eliminate P. In fact, hydrophilic polymers, like the adhesives used in this study, function as permeable membranes that allow the movement of water through the dentin even after polymerization. Thus, universal adhesives may not have the capacity to seal the dentin and can cause some postoperative sensitivity.

In this methodology, the variables related to the tooth itself—such as the regional differences in dentin P,[48] dentin sclerosis,[49] or aging[50]—are beyond the researcher's control. Nevertheless, other variables are controlled by the researcher and need to be set before running the study. The adhesive manufacturers' instructions are well designed; however, they are not very detailed and have some level of ambiguity. For this reason, an effort was made to specify each step to the greatest extent possible to ensure some degree of standardization of the protocol (Table 1).

Although the differences in dentin P among the experimental groups are statistically significant, it does not necessarily mean that they are also clinically significant. It is important to note that when interpreting laboratory research results, one must be aware that the main goal is to study specific variables and compare them to what could be the perfect result. With that critical observation in mind, however, it can be expected that the existence of statistically significant differences has a higher probability of corresponding to clinically significant differences than their absence. Additional clinical studies are essential to further evaluate the performance of these adhesives because clinical trials remain the best way to collect scientific evidence on the effectiveness of an adhesive.

CONCLUSIONS

The results of this study require the rejection of the null hypotheses. No adhesive system, regardless of the application mode, was able to completely eliminate dentin P. It was observed that the differences in P reduction by the application mode were dependent on the adhesive system used. For OPT and ADH, the P reduction was higher in the ER mode than in the SE mode. For CL and SBU, no differences in P reduction were observed between the 2 application modes. All the adhesive systems showed nanoleakage, and the nanoleakage patterns were different depending on the adhesives used.

Regulatory Statement

The teeth were gathered after obtaining informed consent under a protocol reviewed and approved by the Ethics Committee, School of Dentistry, Universidade de Lisboa.

Conflict of Interest

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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