

Effect of Desensitizing Agents on the Bond Strength of Mild and Strong Self-etching Adhesives

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Clinical Relevance

When a desensitizing agent is indicated prior to bonding, clinicians should be aware of its potential effect on the bond strength to dentin.

SUMMARY

Background: Desensitizing agents are used, almost as routine practice, in many adhesive restorative procedures. There is still debate as to their effect in dentin bonding, particularly with self-etching adhesives. The present study aimed to evaluate the effect of different desensitizing agents on the bond strength of mild and strong self-etching adhesive systems to dentin.

Materials and Methods: One hundred twenty recently extracted, noncarious human molars were used to obtain superficial dentin substrate for bonding. No desensitizer was used in the control groups. The experimental groups were pretreated with Gluma Desensitizer, MicroPrime B, and Dentin Desensitizer immediately prior to bonding with self-etching adhesives Optibond XTR, Xeno IV, and iBond. A bonding jig was used to fabricate composite cylinders, which were stored for either 24

hours or three months, after which the shear bond strength (SBS) was evaluated using a notched-edge testing device at a crosshead speed of 1 mm/min. Failure mode distribution was also evaluated at 24 hours and three months. A two-way analysis of variance, Tukey test, and Student *t*-test, with a significance level of $p < 0.05$, were used for data analysis.

Results: At 24 hours, there was no significant difference in SBS when the same adhesive was used with any of the experimental desensitizing agents compared with the control group without desensitizer. However, at three months, Dentin Desensitizer bonded with Optibond XTR demonstrated significantly lower SBS ($p < 0.001$), while Gluma bonded with iBond showed significantly higher SBS values ($p = 0.034$) relative to their corresponding control group. Only MicroPrime B bonded with Xeno IV and iBond with no desensitizer demonstrated a significant reduction in SBS after three months ($p = 0.034$ and $p = 0.002$, respectively). The most prevalent type of failure was adhesive.

Conclusion: Desensitizing agents can be used in combination with self-etching adhesives to control hypersensitivity without adversely affecting their bond strength to dentin.

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INTRODUCTION

Dentinal sensitivity is a painful condition that affects between 10% and 20% of the population.¹ Its etiology may involve tooth wear, gingival recession, tooth bleaching, and many restorative procedures. In particular, postoperative sensitivity is often observed as an undesirable outcome to the placement of resin composite restorations.^{2,3} The prevalence of postoperative sensitivity following application of bonded resins has been reported to range between 0% and 50%.⁴⁻⁶

The two approaches used today in adhesive dentistry for the placement of resin composite restorations are etch-and-rinse and self-etch.⁷ The former uses 32%-37% phosphoric acid etchant prior to infiltration with resin monomers, whereas the latter uses self-etching primers. While phosphoric acid dissolves the smear layer and opens dentin tubules for infiltration with resin monomers, self-etching primers partially dissolve hydroxyapatite, modifying the smear layer rather than dissolving it and thus becoming part of the hybrid layer.^{8,9} In addition to being regarded as less technique sensitive, self-etching adhesives are also known to yield lower postoperative sensitivity compared with etch-and-rinse systems.⁸ This is largely the result of the less aggressive demineralization pattern and thus the more superficial interaction with dentin, which leaves tubules largely obstructed with smear minimizing water movement across the interface.^{8,10,11} However, postoperative sensitivity is still a relatively common finding with self-etching adhesives, perhaps because of the continued action of the acidic monomers, which causes further demineralization beyond the depth of adhesive resin infiltration, leaving areas of unencapsulated collagen at the bottom of the hybrid layer where fluid movement can still occur.¹²

Of the different theories put forward to explain the mechanism of dentin hypersensitivity, the most widely accepted is Brännström's hydrodynamic theory. A stimulus acts on the open tubules of the exposed dentin increasing the rate of dentinal fluid flow and generating action potentials in intradental nerves, which are passed on to the brain, generating pain.¹³ Treatment strategies for dentin hypersensitivity may include the use of neural stimulus blockers, anti-inflammatory drugs, protein precipitants, tubule-occluding agents, and lasers.^{14,15} The use of topically applied dentin desensitizers is a common and effective method to treat dentin hypersensitivity. Different agents have been used for this purpose, including oxalates, which create tubule obstruction by precipitating fine-grained

calcium oxalate crystals¹⁶⁻¹⁸; dentin adhesives^{19,20}; and protein-precipitating fixative agents.^{21,22} Widely used desensitizing components include fluoride (Fluoridin N5, VOCO, PrepEze, Pentron Clinical, Orange, CA, USA), glutaraldehyde/hydroxyethyl methacrylate (HEMA; Gluma, Heraeus Kulzer GmbH, MicroPrime B, Danville Materials, San Ramon, CA, USA), and oxalate (BisBlock, Bisco, Schaumburg, IL, USA). According to Porto and others,²³ desensitizers containing glutaraldehyde/HEMA are considered the first treatment choice for dentinal hypersensitivity. While glutaraldehyde is a biological fixative known to cause coagulation of plasma proteins in the dentin fluid physically blocking dentinal tubules,²⁴ HEMA physically blocks the dentinal tubules.²⁵ Morphological and clinical studies with Gluma desensitizer, an aqueous solution of 5% glutaraldehyde (GA) and 35% HEMA, have shown peripheral tubular blockage and significant pain relief following topical application to hypersensitive dentin.^{21,22} Moreover, Gluma desensitizer has also been shown to either maintain^{26,27} or improve dentin bond strength.²⁸⁻³⁰

Restorative treatment is often indicated in conjunction with the use of desensitizing agents.²³ Moreover, the use of desensitizing agents has been incorporated, almost as a routine procedure, in most adhesive restorative procedures irrespective of the bonding approach.³¹ However, the effect of desensitizers on bond strength is still controversial. A few studies have reported positive or no effect^{27,29,32-35} while others have shown a negative effect^{27,33,34} on the bond strength when desensitizing agents were incorporated into the bonding sequence. Despite the benefits associated with their use, the bond performance may be affected, compromising the integrity and longevity of adhesive restorations. Hence, evaluation of the effect on different desensitizing agents on the bond performance of various adhesive systems is needed to understand the benefit-risk ratio associated with their use when combined with different adhesive systems.

Hence, the objective of this *in vitro* study was to evaluate the effect of different desensitizing agents on the shear bond strength (SBS) of mild and strong self-etching adhesive systems to dentin. The null hypothesis was that the desensitizing agents would have no effect on the SBS to dentin of the different self-etching adhesive systems at 24 hours and three months.

METHODS AND MATERIALS

One hundred twenty recently extracted, noncarious human molars were used to obtain superficial dentin

Table 1: Study Materials: Composition and Application Protocol as per the Manufacturer's Description

Material	Manufacturer	Lot No.	pH	Composition	Application Protocol
Optibond XTR	Kerr Corp	3565224	2.5	Primer: Acetone (25%-35%), ethyl alcohol (4%-15%), HEMA (30%-50%) Adhesive: Ethyl alcohol (20%-30%), alkyl dimethacrylate resins (47%-68%), barium aluminoborosilicate glass (5%-15%), fumed silica (silicon dioxide; 3%-10%), sodium hexafluorosilicate (0.5%-3%)	<ul style="list-style-type: none"> • Apply primer and scrub for 20 s • Air thin for 5 s with medium air pressure • Apply adhesive and scrub for 15 s • Air thin for 5 s • Polymerize for 10 s
iBond	Heraeus Kulzer	010107	1.7	Acetone (25%-50%), 4-META (10%-25%)	<ul style="list-style-type: none"> • Apply iBond SE and scrub for 20 s • Carefully air dry for 5-10 s or until surface appears glossy • Polymerize for 20 s (with 400-500 mW/cm²)
Xeno IV	DENTSPLY Caulk	100111	2.3	Acetone (<50%), UDMA (<15%), dipentaerythritol pentaacrylate phosphate (<15%), polymerizable dimethacrylate resin (<10%), polymerizable trimethacrylate resin (<10%), polymerizable dimethacrylate resin (<10%)	<ul style="list-style-type: none"> • Apply XIV in two coats and scrub for 15 s • Gently air dry for 5 s • Polymerize for 10 s
Gluma Desensitizer	Heraeus Kulzer	010094	1.8	HEMA (25%-50%) Glutaraldehyde (5%-10%) Water	<ul style="list-style-type: none"> • Apply desensitizer and let it sit for 30-60 s • Dry surface until fluid film disappears • Apply adhesive resin
MicroPrime B	Danville Materials	17683	3.6	HEMA (25%-45%) BAC (1%-5%) Sodium fluoride (10 ppm)	<ul style="list-style-type: none"> • Apply desensitizer with microbrush and let it sit for 30 s • Air dry or leave moist • Apply adhesive resin
Dentin Desensitizer	Pulpdent	100721	5.6	Glutaraldehyde (5%) Sodium fluoride (1%) Water	<ul style="list-style-type: none"> • Apply desensitizer for 20-30 s • Blot or apply short blast of air to remove excess, but do not dry • Apply adhesive resin
Abbreviations: 4-META, 4-methacryloxyethyl trimellitic acid anhydride; BAC, benzalkonium chloride; HEMA, 2-hydroxyethyl methacrylate; UDMA, urethane dimethacrylate. ^a					

substrate for bonding. The teeth were obtained under a protocol approved by the State University of New York's Institutional Review Board and stored in a 0.5% Chloramine-T solution at 4°C before use. The crowns were separated from the roots with a slow-speed diamond saw and embedded in a chemically polymerized methacrylate (Fastray, HJ Bosworth, Skokie, IL, USA) with the facial surface exposed and ground flat on a model trimmer to reveal superficial dentin, which was finished with 320-, 400-, and 600-grit silicon carbide abrasive paper (Buehler, Evanston, IL, USA). The specimens were stored in deionized water at 4°C until ready to be used. One hour prior to bonding, the specimens were acclimatized to room temperature (23 ± 2°C) and refinished with 600-grit abrasive paper to expose fresh dentin.

Self-etching adhesive systems, Optibond XTR (Kerr Corporation, Orange, CA, USA), iBond (Heraeus Kulzer GmbH, Hanau, Germany), and Xeno IV (DENTSPLY Caulk, Milford, DE, USA) were used in combination with the desensitizers Gluma Desensi-

tizer (Heraeus Kulzer GmbH), MicroPrime B (Danville Materials), and Dentin Desensitizer (Pulpdent Corp, Watertown, MA, USA) for bond strength evaluation at 24 hours and three months. The composition and application protocol for all the materials evaluated in this study, as recommended by their manufacturer, are summarized in Table 1. Each adhesive system was used either with no desensitizer (control) or in combination with one of three desensitizers for a total of 12 groups with a sample size of 20 per group. Two hundred forty specimens were equally and randomly assigned to the 12 groups as follows: 1, Optibond XTR + Gluma; 2, Optibond XTR + MicroPrime B; 3, Optibond XTR + Dentin Desensitizer; 4, Optibond XTR alone (control); 5, iBond + Gluma; 6, iBond + MicroPrime B; 7, iBond + Dentin Desensitizer; 8, iBond alone (control); 9, Xeno IV + Gluma; 10, Xeno IV + MicroPrime B; 11, Xeno IV + Dentin Desensitizer; 12, Xeno IV alone (control).

After dentin pretreatment with or without desensitizer, the corresponding adhesives were applied

Table 2: Mean (SD) Shear Bond Strength Results for the Different Combinations of Adhesive and Desensitizing Agent at 24 hours (n=10)

	Gluma	MicroPrime B	Dentin Desensitizer	Control
Optibond XTR	34.5 (9.8) ^{A,a}	44.2 (10.1) ^{A,a}	35.4 (11.7) ^{A,a}	39.1 (8.4) ^{A,a}
iBond	34.9 (9.7) ^{A,a}	29.4 (11.8) ^{A,b}	21.7 (6.6) ^{A,b}	33.7 (9.1) ^{A,a*}
Xeno IV	36.9 (21.0) ^{A,a}	41.8 (11.3) ^{A,a,b*}	31.1 (12.2) ^{A,a,b}	40.6 (15.0) ^{A,a}

The same superscript letter indicates no significant differences among groups (Tukey test; $p < 0.05$). Uppercase denotes differences among desensitizers for each adhesive (horizontal). Lowercase denotes differences among adhesives for each desensitizer (vertical)
 * Groups that were significantly different from their corresponding three-month values (Student t-test; $p < 0.05$).

and polymerized, as per each of their manufacturer's instructions, with an LED light-curing unit (Blue-phase 16i, power density 1600 mW/cm², Ivoclar-Vivadent, Amherst, NY, USA) in high-intensity mode. The specimens were stabilized on a bonding jig (Ultradent, South Jordan, UT, USA) with a cylindrical mold of standardized dimensions (2.38 mm in diameter and 2 mm in height). Composite cylinders were fabricated with resin composite (Filtek Z100, Lot No. N196007, 3M ESPE, Saint Paul, MN, USA) in shade A2 by application of only one increment no greater than 2 mm and polymerized for 20 seconds. The specimens were stored in distilled water containing 0.02% sodium azide at 37°C for either 24 h or three months, after which SBS was evaluated. A calibrated testing device (UltraTester, Ultradent) loaded at a crosshead speed of 1 mm/min and a load cell of 1000 lb (453.6 kg) was used. A notched-edge crosshead matching the diameter of the bonded cylinder was used to apply the testing load. The load required to debond the specimen was recorded and expressed in megapascals (MPa) and the group means calculated.

Individual two-way analysis of variance (ANOVA) tests were conducted to evaluate the effect of the main variables adhesive and desensitizing agent as well as their interactions on the bond strength at 24 hours and three months. A post hoc multiple-comparisons Tukey test was used for pairwise comparisons between group means. Student *t*-tests were used to evaluate differences between 24 hours and three months for each of the individual combinations. A significance level of $p < 0.05$ was used for all tests. All statistical analyses were performed with SigmaStat version 3.5 (San Jose, CA, USA). Analysis of the failure mode was conducted through observations, by a single trained evaluator (Z.W.), with a field emission scanning electron microscope in secondary electron and backscattered modes (Hitachi SU-70, Hitachi, Krefeld, Germany) at a magnification of 50×. The failed interfaces were classified as adhesive (A), cohesive in dentin (D), cohesive in composite (C), or mixed (M).³⁶ Mixed failure was

defined as the combination of different failure modes resulting from failure across the interfacial layers.

RESULTS

Two-way ANOVA revealed a significant effect of the variables, adhesive and desensitizing agent, on the bond strength both at 24 hours ($p = 0.003$ and $p = 0.016$) and three months ($p < 0.001$ and $p < 0.001$). The interaction between these variables was significant only at three months ($p = 0.002$). The ranking of the groups, from highest to lowest SBS values, was the same whether it was evaluated at 24 hours or three months as follows: MicroPrime B > Gluma > Dentin Desensitizer and Optibond XTR > Xeno IV > iBond. Mean (SD) SBS values for the different combinations of adhesive and desensitizing agent at 24 hours and three months are summarized in Tables 2 and 3, respectively. In general, the use of desensitizing agents yielded no differences in SBS values relative to the control without desensitizer. Only two groups, when evaluated after three months, demonstrated significant variations from the control group. Dentin Desensitizer bonded with Optibond XTR showed significantly lower SBS values ($p < 0.001$), and Gluma bonded with iBond showed significantly higher SBS values ($p = 0.034$) relative to the control group (Table 3). MicroPrime B bonded with Optibond XTR demonstrated the highest mean SBS values of all groups both at 24 hours and three months (44.2 and 46.9 MPa, respectively). These values, however, were not significantly different from their corresponding control groups with no desensitizer.

Student *t*-test for each of the individual combinations of adhesive and desensitizing agent revealed no significant differences after three months of storage relative to their 24-hour values, with the exception of MicroPrime B bonded with Xeno IV and iBond without desensitizer, which demonstrated a significant decrease in SBS after three months ($p = 0.034$ and $p = 0.002$, respectively). The percentage bond variation after three months relative to 24 hours is also summarized in Table 3.

Table 3: Mean (SD) Shear Bond Strength Results for the Different Combinations of Adhesive and Desensitizing Agent at Three Months (n=10)				
	Gluma	MicroPrime B	Dentin Desensitizer	Control
Optibond XTR	37.3 (13.8) ^{A,B,a}	46.9 (5.5) ^{A,a}	27.4 (9.4) ^{B,a}	43.9 (7.7) ^{A,a}
	8.1%	6.0%	-22.5%	12.1%
iBond	32.4 (10.2) ^{A,a}	31.8 (9.4) ^{A,B,b}	25.7 (7.7) ^{A,B,a}	21.2 (6.3) ^{B,b*}
	-7.1%	8.0%	18.4%	-37.1%
Xeno IV	29.4 (9.7) ^{A,a}	31.4 (8.7) ^{A,b*}	27.7 (9.4) ^{A,a}	34.9 (8.7) ^{A,a}
	-20.4%	-24.8%	-10.8%	-13.9%
Percentage bond variation after three months relative to 24 hours. Positive percent values denote bond strength increase. Negative percent values denote bond strength decrease. The same superscript letter indicates no significant differences among groups (Tukey test; p<0.05). Uppercase denotes differences among desensitizers for each adhesive (horizontal). Lowercase denotes differences among adhesives for each desensitizer (vertical). * Groups that were significantly different from their corresponding 24-hour values (Student t-test; p<0.05).				

The failure mode distribution for specimens fractured at 24 hours and three months is summarized in Figures 1 and 2, respectively. Overall, the most prevalent failure mode was adhesive in nature. Relative to 24 hours, the number of adhesive failures increased for all groups after three months. Cohesive fractures in dentin were also observed, particularly at 24 hours, but were fewer in number. A few cohesive fractures in composite were shown at 24 hours only but not after three months. No correlations were observed between bond strength values and mode of failure.

DISCUSSION

The present study investigated the effect of different desensitizing agents on the SBS of mild and strong self-etching adhesives. The null hypothesis was only partially rejected as the desensitizing agents generally showed no effect on the SBS to dentin of the

different self-etching adhesive systems at 24 hours or three months. Only two exceptions were observed at three months: Dentin Desensitizer bonded with Optibond XTR, which showed lower SBS than the control, and Gluma bonded with iBond, which showed higher SBS than the control. Our results are in agreement with previous studies,^{26,27} which have shown that desensitizing agents can be safely incorporated into the bonding sequence without adversely affecting the bond performance. Despite the lack of significant variations relative to their corresponding control group, certain combinations seemed more favorable than others. Optibond XTR demonstrated the best performance of the three adhesives. After three months, when Optibond XTR was used either alone or in combination with Gluma or MicroPrime B, an increase in bond strength was shown. We speculate that this may have been the

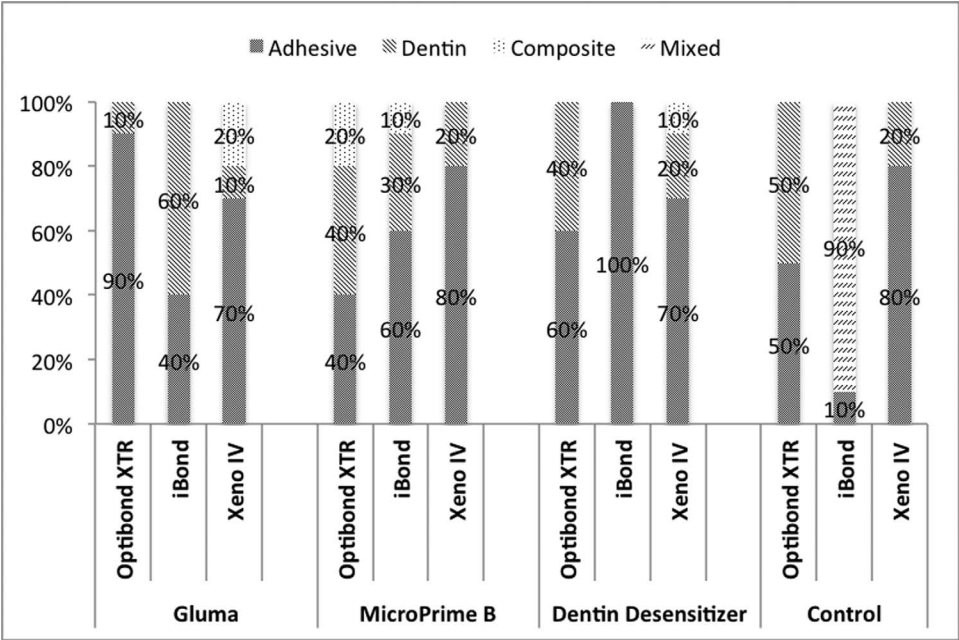


Figure 1. Failure mode distribution for the different combinations of adhesive and desensitizing agent at 24 hours (n=10). Modes of failure described as adhesive (A), cohesive in dentin (D), cohesive in composite (C), and mixed (M).

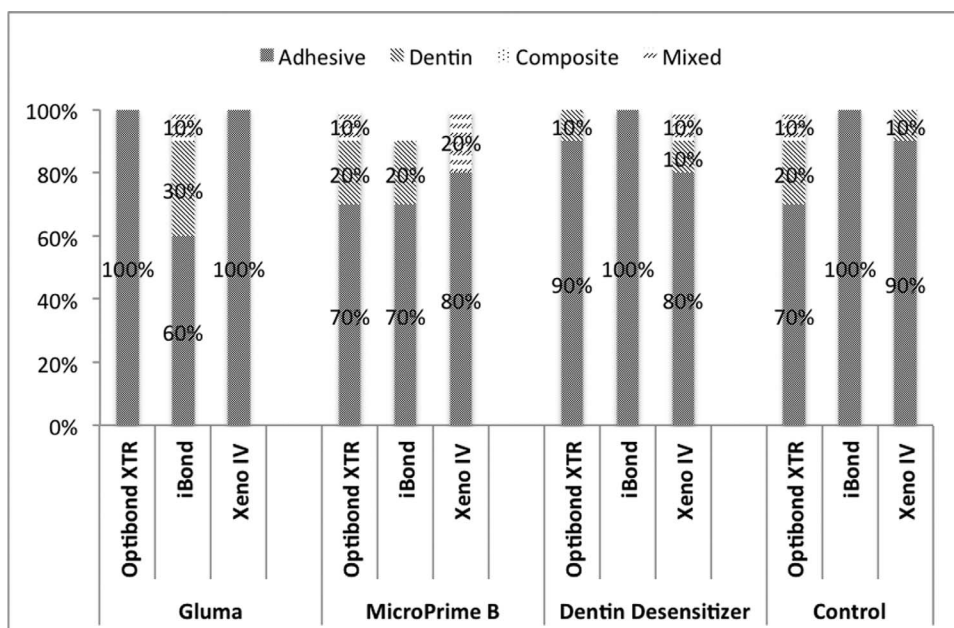


Figure 2. Failure mode distribution for the different combinations of adhesive and desensitizing agent at three months ($n=10$). Modes of failure described as adhesive (A), cohesive in dentin (D), cohesive in composite (C), and mixed (M).

result of compatibility aspects between materials, as well as their individual composition and pH. Optibond XTR film thickness is in the range of 5 to 10 μm . Its reduced film thickness, which improves its wetting properties, in combination with its hydrophilic nature, which keeps collagen from collapsing, may have contributed to a greater monomer infiltration and consequently to a longer resin tag formation.

Three self-etching adhesives were used in this study, which were different in composition, pH, and their bonding approach. While Optibond XTR is a two-step system, including the self-etching primer, and mostly solvent free, the hydrophobic adhesive resins, iBond and Xeno IV, are all-in-one systems, combining acidic, hydrophilic, and hydrophobic monomers with organic solvents and water into a single bottle. All-in-one systems are known to form highly hydrophilic polymers that behave as permeable membranes, allowing diffusion of water from dentin across the hybrid and adhesive layers.³⁷ This may help explain the overall superior performance of the two-step system, Optibond XTR, relative to all-in-one adhesives, Xeno IV and iBond. In general, self-etching adhesives can be classified as ultra-mild ($\text{pH}>2.5$), yielding demineralization of only a few hundreds of nanometers; mild ($\text{pH}\sim 2$), with demineralization of about 1 μm ; intermediately strong ($\text{pH}=1-2$), with demineralization about 1-2 μm ; and strong ($\text{pH}\leq 1$), with demineralization greater than 2 μm . Only with intermediately strong and strong self-etching adhesives can typical resin tags be formed,

while they are hardly formed with mild and ultra-mild self-etching adhesives.³⁸ The interfacial structure of highly acidic self-etching adhesives resembles that of etch-and-rinse systems, with the difference that the dissolved mineral phase is not rinsed away. These calcium phosphates are known to be very unstable, greatly contributing to weakening the interface.³⁸ Moreover, as the acidity of the adhesive increases, the issues with water permeability also become more acute, leaving water-filled nano-spaces at the interfacial layer. Water contributes to both degradation of collagen fibrils and composite plasticization, leading to the accelerated destruction of the hybrid layer and the consequent loss of the dentin bond strength over time.³⁸ Of the adhesives evaluated in our study, Optibond XTR ($\text{pH } 2.5$) and Xeno IV ($\text{pH } 2.3$) can be considered ultra-mild and mild, respectively, whereas iBond can be considered intermediately strong. The greater acidity of iBond may help explain its overall lower performance relative to Xeno IV and Optibond XTR. Only when iBond was bonded with Gluma and evaluated after three months was a significantly higher bond strength relative to the control group seen.

Because of the water content in these mixtures, desensitizing agents also serve as rewetting agents that expand the demineralized collagen network and increase its surface energy, all of which facilitate the diffusion of resin monomers into the partially demineralized dentin, thus improving resin-dentin bonds.³⁹ Hydroxyethyl methacrylate, a water-soluble monomer present in most dental adhesives and

in two of the three desensitizers evaluated, is known to improve infiltration of the partially demineralized collagen because of its ester and hydroxyl functional groups and its hydrophilic nature.⁴⁰ Because of its known role in facilitating diffusion of resin monomers into the partially demineralized collagen network, HEMA is considered an adhesion promoter.⁴¹ Moreover, strong inhibitory properties of matrix metalloproteinase MMP-2⁴² and MMP-9⁴³ have also been shown with monomeric HEMA. However, the frequently reported fall in bond strength of HEMA-containing adhesives^{44,45} indicates that the MMP-inhibitory effects of HEMA may be lost when it is copolymerized with other adhesive monomers.⁴³ Overall, Dentin Desensitizer, the only HEMA-free desensitizer evaluated in our study, demonstrated lower performance than HEMA-containing desensitizers, Gluma and Micro-Prime B. Of the three desensitizers evaluated, MicroPrime B demonstrated the best overall performance when the data were combined. When the data were analyzed separately at 24 hours and three months, MicroPrime B was also shown to perform best, particularly when combined with Optibond XTR and Xeno IV. MicroPrime B contains benzalkonium chloride (BAC) and sodium fluoride in addition to HEMA. As a surface surfactant, BAC may allow for a more interactive surface. Conversely, the desensitizing mechanism of sodium fluoride is derived from the formation of precipitates, which mechanically block the exposed dentinal tubules.⁴⁶ Studies have shown a reverse relation between the amount of fluoride in the desensitizing agent and dentin bond strength.⁴⁷ This may be the result of crystal precipitation, which may physically prevent penetration of the adhesive resin monomers.^{48,49} Availability of calcium ions on the dentin surface is critical to the formation of precipitates, suggesting that less precipitation will occur in a more acidic environment. Moreover, fluoride salts are soluble at lower pH values, making crystal precipitates more likely to remain intact when in a mildly acidic environment such as the one created by Dentin Desensitizer (pH=5.6) and to a lesser extent MicroPrime B (pH=3.6). Application of a highly acidic overlaying self-etching adhesive may further dissolve these precipitates.

The common active component of Gluma and Dentin Desensitizer is 5.0% glutaraldehyde. Of the two agents, Gluma demonstrated better overall performance. Gluma has been used since 1991 as a dentin desensitizer.⁵⁰ Its desensitizing mechanism remained elusive until Schüpbach and others showed that topical application *in vivo* of glutaral-

dehyde/HEMA combination products resulted in a series of horizontal partitions within the lumens of exposed dentinal tubules.²² More recent spectrophotometric studies *in vitro*²⁵ revealed that the glutaraldehyde in Gluma reacts with plasma proteins such as albumin to form protein precipitates, which then react with HEMA to form a mixture of poly-HEMA copolymerized with glutaraldehyde-cross-linked albumin.⁵¹ These precipitates occlude open dentinal tubules beneath the surface, which interferes with the hydrodynamics of dentinal fluid, thereby preventing dentin sensitivity. It remains unclear how long these precipitates stay in dentinal tubules. Dentinal fluid and saliva contain esterases⁵² that could attack the ester and peptide bonds in these intratubular precipitates.⁵³ Saliva also contains MMPs⁵⁴ and kallikreins⁵⁵ that could attack collagen and plasminogen, respectively. If these enzymes attacked the Gluma-created precipitates, their desensitizing activity would be lost if the intratubular precipitates were destroyed. Glutaraldehyde is also a potent antimicrobial and cross-linking agent known to improve the resistance of un-cross-linked or mildly cross-linked collagen matrices to enzymatic degradation by collagenases.^{56,57} The mechanism seems to be dependent on the reaction between the aldehyde group of glutaraldehyde and the amino groups of lysine and hydroxylysine residues in collagen^{58,59} that increase the resistance of collagen to enzymatic degradation.⁵⁹ By improving dentin's mechanical properties, glutaraldehyde can also minimize the degradation of the resin-dentin bonds.

In general, no significant differences were shown among adhesives, desensitizers, or after storage, so speculations have been provided based on trends only. Although it was not the intent of our study to evaluate the degradation patterns of the interfaces created with different combinations of materials, the drop in bond strength values observed for some groups after only three months was unexpected and caused concern. Future bond degradation studies are thus necessary to confirm these trends. Only MicroPrime B bonded with Xeno IV and iBond without desensitizer demonstrated a significant bond strength reduction after three months, with a 24.8% and 37.1% drop, respectively. Groups such as Dentin Desensitizer bonded with Optibond XTR and Gluma bonded with Xeno IV, with a drop in bond strength values of 22.5% and 20.4%, respectively, although not significantly different from their corresponding 24-hour values, were still of concern. The positive percentage variation values

in Table 3 indicate groups whose values increased after three months. Although these differences were not significant, they may indicate further maturation of the bonds. A postirradiation polymerization reaction takes place after initial irradiation, and it is known to extend to the first 24 hours.⁶⁰ Increased bond strength values, however, may suggest further maturation of the bonds past the initial 24 hours. Since the acidity of self-etching adhesives continues to demineralize the dentin matrix beneath the hybridized layer,⁶¹ we speculate that further diffusion into the matrix and chemical bonding of some resin monomers in these groups may have continued to strengthen the hybrid zone.

Although our results are limited to the systems and techniques evaluated in this study and cannot be extrapolated to draw conclusions as to the behavior of self-etching adhesives when used in combination with different desensitizing agents, they suggest the use of desensitizing agents does not affect initial bond performance. Future bond degradation studies with a larger sample size and longer incubation times are needed to identify differences in degradation patterns when using different combinations of adhesives and desensitizing agents. Moreover, micro-tensile bond strength tests may be recommended for bond degradation studies since long incubation periods, between 2 and 4 years, may be necessary to detect the effects of hydrolytic degradation for specimens of a large surface area such as those used in SBS tests due to the required longer diffusional distances from the cavosurface margin.⁶² When compared with micro-tensile tests, SBS tests are also known to be less discriminating in their ability to detect differences, perhaps requiring a considerably larger sample size to be able to detect differences. One of the limitations of the present study may have been the sample size of 10, which may have limited our ability to detect differences. Further evaluation of different combinations of adhesives and desensitizing agents, as well as evaluation of desensitizers with different mechanisms of action, should precede the adoption of clinical techniques involving incorporation of desensitizing agents in the bonding sequence.

CONCLUSIONS

Within the limitations of this *in vitro* study, it can be concluded that the effect of desensitizing agents on the SBS of self-etching adhesives to dentin appears to be material and time dependent. This indicates

that they may be safely incorporated in the bonding sequence of self-etching systems without adversely affecting their bond performance.

Acknowledgment

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Conflict of Interest

The authors 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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