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Laboratory Research

Effect of a Chlorhexidine-containing Adhesive on Dentin Bond Strength Stability

C Sabatini

Clinical Relevance

A novel chlorhexidine-containing adhesive may offer comparable dentin-resin bond stability to the use of 2.0% chlorhexidine digluconate applied as a therapeutic primer while also providing a simplified clinical application technique.

SUMMARY

Purpose: The present study aimed to investigate a novel adhesive system containing 0.2% chlorhexidine digluconate (CHX) for its ability to improve the stability of the adhesive interface compared with the use of 2% CHX as a therapeutic primer. Furthermore, the study aimed to confirm the inhibitory properties of these CHX concentrations (0.2% and 2.0%) on dentin matrix metalloproteinase activity by gelatin zymography.

Methods: Superficial dentin substrate for bonding was obtained from 120 non-carious human molars. A conventional adhesive Peak LC Bond and a CHX-containing adhesive Peak Universal Bond were used either in combination with 35% phosphoric acid (etch-and-rinse approach) or with self-etching primer (self-etch approach) for evaluation of the variables CHX treatment (2.0% therapeutic primer and 0.2% adhesive), adhesive approach (etch-and-rinse and self-etch), and storage time (24 hours and six months). A bonding jig was used to fabricate composite cylinders, which were stored for either 24 hours or six months, after which shear bond strength (SBS) was evaluated using a notched-edge testing device. A three-way analysis of variance and a Student *t*-test with a significance level of $p<0.05$ were used to analyze the data. Extracts from concentrated demineralized human dentin powder were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis and incubated in the presence of 0.2% and 2.0% CHX.

Results: No significant effect of CHX treatment, adhesive approach, storage time variables, or their interactions on mean SBS was

*Camila Sabatini, DDS, MS, assistant professor, University at Buffalo, Restorative Dentistry, Buffalo, NY, USA

*Corresponding Author: 3435 Main St, 215 Squire Hall, Buffalo, NY 14214; e-mail: cs252@buffalo.edu

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demonstrated ($p<0.05$). No significant difference between the control and the CHX-treated groups was detected for either adhesive technique at 24 hours or six months ($p<0.05$). No significant variation in mean SBS was detected after six months of storage ($p<0.05$). Zymographic analysis revealed bands of enzymatic activity for the group demineralized with phosphoric acid and complete inhibition of gelatinolytic activity for the groups treated with 0.2% and 2.0% CHX.

Conclusions: CHX demonstrated inhibition of dentin proteolytic activity. However, when CHX was incorporated into a commercially available adhesive or used as a therapeutic primer, no difference in bond strength was observed at baseline or after six months of storage relative to the control group without CHX.

INTRODUCTION

Despite improvements made to the chemistry of contemporary dentin adhesives, resin-dentin interfaces still deteriorate over time.[1-7] A correlation between the presence of active endogenous matrix metalloproteinases (MMPs) in dentin matrices and premature degradation of hybrid layers has been demonstrated.[8,9] Different forms of MMPs have been identified in dentin matrices by zymographic analyses, with MMP-2 and MMP-9 being the most prevalent.[8,10,11] When exposed to an acidic environment, such as the one created by the application of acidic adhesive resins, these enzymes, initially secreted as pro-enzymes, become active proteinases.[12] These host-derived MMPs have been shown to degrade sub-optimally infiltrated collagen fibers once they have been activated through bonding procedures.[9,13]

Currently, bonding to dentin can be achieved through an etch-and-rinse (ER) or a self-etch (SE) approach. Etch-and-rinse adhesives have a pH too high (2.5-4.5) to etch through the smear layer and underlying dentin, hence the requirement for a separate acid-etching step with 32%-37% phosphoric acid (0.1-1.0). Subsequent infiltration of the resin monomers into the demineralized dentin matrix typically leaves collagen incompletely encapsulated at the bottom of the hybrid layer,[14] which is then susceptible to proteolytic degradation by host-derived MMPs. Self-etch adhesives, with a higher pH (1.0-2.7), can still etch through the smear layer. Despite the shallow etching pattern, optimal hybridization and high bond strengths have been reported with these formulations.[15] Activation of precursor MMP forms has been demonstrated with both ER[16] and SE[12, 13] adhesive systems.

Chlorhexidine digluconate (CHX), a cationic antimicrobial agent, has demonstrated successful inhibition of dentin MMP collagenolytic activity in zymographic studies when used in concentrations of 0.2% and 2.0 %.[17-19] Clinically, reduced degradation of the dentin-adhesive interface has been shown with the use of 2% CHX as a therapeutic primer before the application of the adhesive *in vivo*[20-24] and *in vitro*.[18,25-28] However, this represents an additional step in the bonding sequence. Recently, alternative approaches incorporating CHX into the phosphoric acid or into the primer/adhesive formulation have been proposed to improve its retention and effectiveness while simplifying the clinical application technique. A study demonstrated reduced bond degradation after six months when 2% CHX was incorporated into a conventional 37% phosphoric acid.[29] Incorporation of CHX into the primer has shown conflicting results of improved stability,[30] but also no effect,[31] on the stability of the adhesive interface after one year. The data on the subject are inconclusive.

Therefore, the objective of the present study was to investigate a novel adhesive system containing 0.2% CHX for its ability to improve the stability of the adhesive interface over time compared with the use of 2.0% CHX as a therapeutic primer. Specific aims of our study included the following: 1) to evaluate dentin shear bond strength (SBS) of the novel adhesive containing 0.2% CHX when applied either as an ER or an SE approach at both 24 hours and six months, compared with the topical application of 2.0% CHX; and 2) to confirm the previously demonstrated inhibitory properties of 0.2% and 2.0% CHX on dentin MMP activity by gelatin zymography. The following null hypotheses were evaluated: 1) there would be no difference in SBS between groups treated with the novel adhesive containing 0.2% CHX, topical 2.0% CHX, and use of no CHX; and 2) there would be no difference in bond degradation after six months for either group.

MATERIALS AND METHODS

Zymographic Analysis

Dentin Extraction—Six healthy human molars from donors of unknown age were obtained under a protocol approved by the State University of New York's Institutional Review Board and stored in a 0.5% NaCl solution containing 0.02% sodium azide

at 4°C for no more than one month after extraction. The crowns were separated from the roots at the cement-enamel junction. Coronal dentin blocks were obtained after complete removal of the enamel and pre-dentin tissues by means of a diamond bur in a high-speed handpiece under air-water spray. A slow-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) was used to obtain smaller dentin sections, which were frozen in liquid nitrogen and triturated into fine dentin powder in a high-speed mixer mill (MM400, Retsch, Newton, PA, USA) at 30 Hz for 10 minutes. Fine dentin powder (4 g) was obtained from six molar crowns. The powder was stored dry at −70°C until ready to use.

Aliquots of 1 g each of dentin powder were randomly assigned to one of four groups: 1) mineralized dentin (control); 2) demineralized dentin; 3) demineralized dentin incubated in 0.2% CHX; and 4) demineralized dentin incubated in 2.0% CHX. Groups 2-4 were demineralized by mixing the dentin powder with a 1% phosphoric acid water solution for 10 minutes. The four groups received five rinses of 1 mL of distilled water to eliminate acid remnants. To extract the protein content from the dentin powder, precipitates were re-suspended in 4 mL of extraction buffer (50 mM Tris-HCL, pH 6.0 with 5 mM $CaCl_2$, 100 mM NaCl, 0.1 mM $ZnCl_2$, 0.1% Triton X-100, 0.1% Triton X-114, 0.02% NaN_3) containing a protease inhibitor free of ethylenediamine tetra-acetic acid (Rosche Diagnostics, Indianapolis, IN, USA) for 24 hours at 4°C per Breschi's protocol.[18] The vials were centrifuged at 8,500 rpm (Sorvall RC 6 Plus Centrifuge, Thermo Scientific, Asheville, NC, USA) for 15 minutes at 4°C, and the supernatants were carefully collected by aspiration. Protein content was precipitated with 25% trichloroacetic acid and centrifuged at 8,500 rpm for 10 minutes at 4°C. The precipitate was stored at −70°C until ready to use for zymographic analysis.

Gelatin Zymography—To assess gelatinolytic activity, concentrated samples from dentin powder protein extracts were electrophoresed under non-reducing conditions on a 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis containing 2g/L gelatin substrate. The precipitates were re-solubilized in loading buffer (2% sodium dodecyl sulfate [SDS]; 125mM Tris-HCL, pH 6.8; 10% glycerol; and 0.001% bromophenol blue), and the samples were separated by electrophoresis for 30 minutes at 300 V, 32 mA, and 6 W. After electrophoresis, the gels were washed in 2.5% Titron X-100 for 15 minutes to remove SDS and activated with 2 mM p-aminophenylmercuric acetate for one hour at 37°C. After activation, Groups 3 and 4 were incubated in aqueous solutions of 0.2% and 2.0% CHX, respectively, for 30 minutes. All groups were incubated in zymography buffer ($CaCl_2$, NaCl, and Tris-HCL, pH 8.0) for 48 hours at 37°C to allow development of enzymatic activity. The gels were then stained in 0.2% Coommassie Brilliant Blue, and de-stained in de-staining solution (50% methanol, 40% acetic acid, and 10% water). Gelatinolytic activity was evidenced as unstained bands on a blue-stained background.

Shear Bond Strength

One hundred and twenty recently extracted, non-carious human molars were used to obtain superficial dentin substrate for bonding. 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). The specimens were stored in deionized water at 4°C until ready to be used. One hour before bonding, the specimens were acclimatized to room temperature (23 ± 2°C) and refinished with 600-grit abrasive paper to expose fresh dentin.

Two adhesive resins were used in this study, a conventional adhesive (Peak LC Bond, Ultradent, South Jordan, UT, USA) and a novel adhesive containing 0.2% CHX (Peak Universal Bond, Ultradent). Both are 7.5 % nanofilled, phosphated, adhesives containing hydroxyethyl methacrylate with ethyl alcohol as the carrier, and can be used either in combination with 35% phosphoric acid (Ultra-Etch, Ultradent) for a two-step ER technique or with self-etching primer (Peak SE primer, Ultradent) for a two-step SE technique. The study variables included CHX treatment (2.0% therapeutic primer and 0.2% adhesive), adhesive approach (ER and SE), and storage time (24 hours and six months). Specimens were equally and randomly assigned to six groups (Table 1) with a sample size of 10 as follows: Group 1, phosphoric acid treatment followed by conventional adhesive (PA+PLC, ER control); Group 2, phosphoric acid treatment followed by topical 2% CHX and conventional adhesive (PA+CHX+PLC); Group 3, phosphoric acid treatment followed by CHX-containing adhesive (PA+PU); Group 4, self-etching primer followed by conventional adhesive (PSE+PLC, SE control); Group 5, topical 2% CHX followed by self-etching

Table 1: *Study Groups, Category, and Application Procedures per Manufacturer Recommendations*[a]

Group	Description	Code	Category/Application Procedure
ER technique			
1	35% H_3PO_4 followed by conventional adhesive resin (control)	PA+PLC	• Apply 35% H_3PO_4 (15 s); rinse (5 s); leave dentin moist • Scrub Consepsis onto moist dentin and air dry (Group 2 only) • Scrub adhesive onto dentin (10 s); gently air dry to leave a thin uniform layer (10 s) • Polymerize (10 s if >600 mW/cm^2; 20 s if <600 mW/cm^2)
2	35% H_3PO_4 followed by rewetting with 2% CHX and conventional adhesive resin	PA+CHX+PLC	
3	35% H_3PO_4 followed by CHX-containing adhesive resin	PA+PU	
SE technique			
4	Self-etching primer followed by conventional adhesive resin (control)	PSE+PLC	• Scrub Consepsis onto moist dentin and air dry (Group 5 only) • Scrub Peak SE primer onto moist dentin (20 s); thin/dry (3 s) • Scrub adhesive onto dentin (10 s); gently air dry to leave a thin uniform layer (10 s) • Polymerize (10 s if >600 mW/cm^2; 20 s if <600mW/cm^2)
5	2% CHX followed by self-etching primer and conventional adhesive resin		
	CHX+PSE+PLC		
6	Self-etching primer followed by CHX-containing adhesive resin	PSE+PU	

Abbreviations: ER, etch-and-rinse; SE, self-etch; CHX, chlorhexidine; PA, phosphoric acid; PLC, Peak LC Bond; PU, Peak Universal Bond; PSE, Peak SE Primer.
[a] *Self-etching primer (Peak SE Primer); conventional adhesive resin (Peak LC Bond); CHX-containing adhesive resin (Peak Universal Bond); aqueous solution of 2% CHX (Consepsis , Ultradent, South Jordan, UT, USA).*

primer and conventional adhesive (CHX+PSE+PLC); Group 6, self-etching primer followed by CHX-containing adhesive (PSE+PU).

The adhesives were applied and polymerized according to manufacturer's instructions with a light-emitting diode light-curing unit (Bluephase 16i, Ivoclar-Vivadent, Amherst, NY, USA) with a power density of 1,600 mW/cm^2. The specimens were placed on a bonding jig (Ultradent) 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, 3M ESPE, Lot# N196007, St 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 hours or six months, after which SBS was evaluated. A calibrated testing device (Ultratester, Ultradent) loaded at a crosshead test speed of 1mm/min and a load cell of 1,000 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 descriptive statistics were determined.

Because the data were normally distributed (Kolmogorov-Smirnov test), a three-way analysis of variance (ANOVA) was used to analyze the effect of the variables CHX treatment, adhesive approach, and storage time. Student *t*-tests were used to evaluate differences between 24 hours and six months for each of the individual groups. A significance level of $p<0.05$ was used for all tests. All statistical analyses were performed with the Statistical Package for Social Sciences (SPSS) version 16.0 (SPSS Inc, Chicago, IL, USA).

Analysis of the Mode of Failure—Modes of failure were analyzed by observation by a single trained examiner (C.S.) with a stereomicroscope (Nikon SMZ-U, Melville, NY, USA) at a magnification of 50×. Representative images of the different failure modes were recorded with a field emission scanning electron microscope (Hitachi SU-70, Hitachi, Kre-

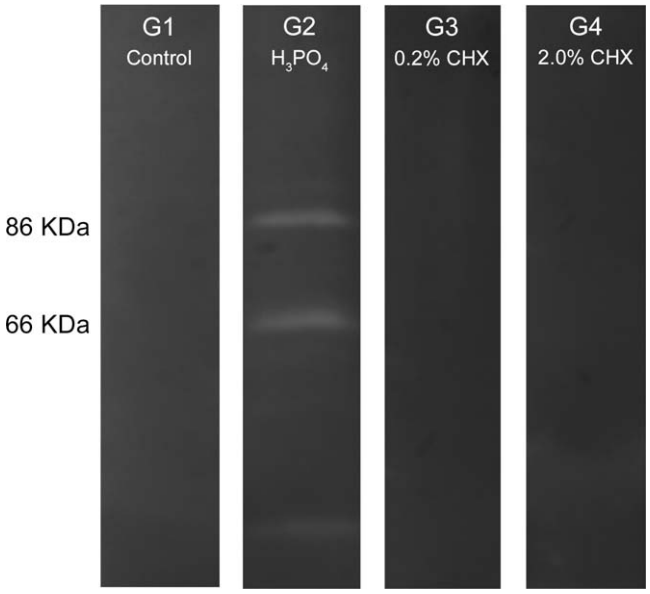

Figure 1. *Gelatin zymogram from concentrated dentin protein extracts. (G1) Mineralized dentin (control). (G2) Demineralized dentin in H_3PO_4 (bands correspond to MMP-2 and MMP-9 isoforms). (G3) Demineralized dentin in H_3PO_4 and incubated in 0.2% CHX. (G4) Demineralized dentin in H_3PO_4 and incubated in 2.0% CHX.*

feld, Germany) in backscattered electron mode at 50×. The fractured surfaces were classified as follows: 1) cohesive in dentin, 2) adhesive, 3) cohesive in composite, and 4) mixed failure, defined as the combination of different failure modes resulting from failure across the interfacial layers.

RESULTS

Gelatin Zymography

When protein extracts were analyzed by gelatin zymography (Figure 1), no activity was detected for the control group with untreated mineralized dentin powder. Bands of enzymatic activity were evidenced for the group that was demineralized with phosphoric acid but received no further treatment with inhibitor (Group 2). These bands corresponded to active MMP-2 and MMP-9 isoforms. Complete inhibition of enzymatic activity was shown with both 0.2% and 2.0% CHX (Groups 3 and 4, respectively).

Shear Bond Strength

Three-way ANOVA (Table 2) revealed no significant effect of the variables CHX treatment, adhesive approach, storage time, and any of their interactions on mean SBS values ($p<0.05$). Table 3 summarizes the mean SBS values and failure mode distribution for all study groups at 24 hours and six months of storage. No significant difference between the control and the CHX-treated groups was detected for either adhesive technique at 24 hours or six months ($p<0.05$). Student *t*-test comparisons between 24 hours and six months for each of the individual groups revealed no significant variation in mean SBS values after six months of storage ($p<0.05$). Mixed-type fracture was the most prevalent type of failure mode observed, irrespective of CHX treatment, adhesive approach, or storage time (Table 3).

DISCUSSION

The present study evaluated the effect of incorporating 0.2 % CHX into a commercially available adhesive blend, used either as an ER or SE approach, compared with the topical application of 2% CHX. Both null hypotheses were accepted as no differences were observed among treatment groups at either testing period; nor were differences in bond degradation observed after six months for each of the individual groups. Because the two main concentrations of CHX evaluated in this study were 0.2% and 2.0%, zymographic analysis was also conducted to confirm the previously demonstrated inhibitory properties of CHX.[17-19] Our results are in agreement with previous studies, which have shown activation

Table 2: *Three-way ANOVA Results*

Source of Variation	DF	SS	MS	F	P
Adhesive	2	15.713	7.856	0.0728	0.930
Composite	1	117.216	117.216	1.086	0.300
Time	1	316.875	316.875	2.936	0.089
Adhesive × composite	2	142.229	71.114	0.659	0.519
Adhesive × time	2	23.994	11.997	0.111	0.895
Composite × time	1	73.320	73.320	0.679	0.412
Adhesive × composite × time	2	185.729	92.864	0.861	0.426
Residual	108	11654.604	107.913		
Total	119	12529.680	105.291		

Abbreviations: DF, degrees of freedom; SS, sum of squares; MS, mean squares; F; f obtained; P*, probability.*

Table 3: *Mean SBS Results and Failure Mode Distribution for the Six Study Groups at 24 hours and Six Months of Storage*[a]

	Group	Storage	SBS (MPa)	Failure mode (%)			
			Mean ± SD	A	D	R	M
ER	1	24 h	40.6 ± 15.7	30	10	0	60
		6 mo	46.4 ± 7.4	0	30	0	70
	2	24 h	40.5 ± 8.5	10	30	0	60
		6 mo	42.2 ± 11.9	0	40	10	50
	3	24 h	46.0 ± 7.2	0	20	0	80
		6 mo	43.6 ± 11.1	0	20	20	60
SE	4	24 h	39.5 ± 9.9	20	30	10	40
		6 mo	42.0 ± 10.5	0	10	10	80
	5	24 h	39.6 ± 11.1	10	20	20	50
		6 mo	45.1 ± 6.4	0	30	10	60
	6	24 h	37.4 ± 11.7	0	30	20	50
		6 mo	43.9 ± 9.8	10	20	0	70

Abbreviations: ER, etch-and-rinse; SE, self-etch; A, adhesive; D, cohesive in dentin; R, cohesive in resin; M, mixed; SBS, shear bond strength.
[a] *No significant differences in mean SBS among groups for either storage period ($p<0.05$). No significant differences in mean SBS between baseline and six months for each of the individual groups ($p<0.05$).*

of endogenous dentin MMPs after treatment with phosphoric acid.[32] Furthermore, both 0.2% and 2.0% CHX demonstrated complete inhibition of dentin proteolytic activity as determined by gelatin zymography validating previous studies.[17-19]

Chlorhexidine, applied either topically or incorporated into the adhesive, did not appear to affect bond strength for either adhesive approach at 24 hours or six months, suggesting that the antimicrobial may be safely combined with the resin monomers contained in the adhesive tested. Our results confirm those from previous studies, which have shown that CHX, applied topically[20,26-28] or into the primer,[30,31] has no effect on the immediate bond strength. Moreover, CHX, applied either topically or into the adhesive, did not appear to affect the stability of the bonds over time, as demonstrated by the non-significant differences in mean bond strength values between 24 hours and six months for each of the individual groups. A number of studies evaluating the stability of adhesive interfaces treated with CHX have demonstrated favorable results of reduced bond degradation.[23-28] However, these studies primarily report on the topical use of CHX before adhesive application; thus, direct comparisons of these results and those from our study are not possible as our study aimed to investigate CHX when incorporated into an adhesive blend rather than topically between the conditioning and adhesive application. Recently, incorporation of CHX into the primer or the adhesive blend has been proposed to optimize its retention and effectiveness while simplifying the clinical application procedures. Conflicting results of improved stability,[30] but also no effect[31] on the stability of adhesive interfaces, have been reported when CHX was admixed into the primer. Similarly, fair comparisons between these results and those from our study are not possible as differences in study methodology, materials evaluated, and primarily in

the actual vehicle for the delivery of CHX vary among studies. The vehicle for the delivery of CHX in our study was the actual adhesive resin rather than the primer. We speculate that this may have limited its interaction with the hybrid layer where MMP inhibition is required. However, even when incorporated into the primer, CHX inhibitory properties may be of limited duration because of its unknown substantivity to dentin. A recent study demonstrated outstanding substantivity of CHX to human dentin[33]; however, results only up to two months of storage were reported in this study, and thus, the long-term role of CHX in the preservation of the bonds requires further investigation when incubated over longer storage periods. Further research is also needed to better understand the anti-proteolytic effects and substantivity properties of CHX and other proposed synthetic inhibitors, so that their use can be optimized, leading to longer-lasting adhesive restorations.

Despite the several benefits of CHX, the inability to stabilize the water soluble, non-covalently bound compound in the adhesive interface may limit its long-term anti-proteolytic benefit.[34] At the same time, polymer plasticization that may further expose collagen fibrils over time requires that MMP inhibiting agents remain available at the interface for effective anti-proteolytic properties over the long-term life of the restoration. Attempts to stabilize CHX into the resin matrix have been proposed by incorporating the compound into the adhesive blend with the belief that the resin matrix can act as a reservoir for the slow release of CHX over time. However, extensive testing of the specific interactions between CHX and the specific adhesive monomers in the different adhesive blends should precede such mixtures, as adverse effects on the adhesive mechanical properties,[35] which may outweigh its anti-proteolytic benefit, may be derived from incorporating CHX into the adhesive blend. Moreover, the nature of the interaction between CHX and adhesive is known to be product specific as it depends on the specific monomeric composition. A study showed that dissolving increasing concentrations of CHX into resin blends with different levels of hydrophobicity decreased their elastic modulus by 27%-48%.[35] Degradation of adhesive interfaces is known to be the combined result of polymer plasticization and collagenolytic activity by host-derived dentin MMPs. Thus, a polymeric network with lower elastic modulus is presumably more susceptible to early plasticization and, as a consequence, to premature failure. Future studies should be undertaken to evaluate which types of resin can be safely combined with this antimicrobial and with other anti-proteolytic agents.

Other aspects may also have contributed to the observed results of non-significant differences between the control and CHX-treated groups in the present study. A storage time of six months may not have been sufficient to detect the effects of hydrolytic degradation of the adhesive interface if the larger surface area of the specimens used for SBS tests is taken into consideration. A study by Kiyomura[36] reported that storage times between 2 and 4 years were required to detect the effects of hydrolytic degradation for specimens of large surface area, such as those used in SBS tests, because of the required longer diffusional distances from the cavosurface margin. Compared with microtensile tests, SBS tests are also known to be less discriminating in their ability to detect differences, perhaps requiring considerably larger sample size to be able to detect differences. A recent review by Braga and others[37] reported that of 100 recently conducted bond strength studies, 59% used a sample size of 10, 15% used a sample size between five and eight, and 26% used a sample size between 11 and 25. In our study, a sample size of 10 was used and no significant differences were detected among groups. A power analysis revealed that a sample size greater than 30 would have been able to detect differences in the range of 4 MPa. However, considering the mean SBS range observed in our study (~40 MPa), a difference of 4 MPa may not be of great clinical relevance. Nonetheless, SBS tests remain useful in the preliminary screening of adhesives, and their simplicity warrants consideration. Useful information can be derived from these data provided that the limitations of the test are understood and its results not overemphasized.

In our study, the most prevalent type of failure mode was mixed type, which, considering the overall high mean bond strength values observed in our study, the type of test, and what is known about the stress distribution created by SBS testing, is not surprising. In general, it is known that in the presence of strong bonds, the fracture path starts in resin composite propagating across the adhesive joint and then into dentin.[38] This suggests that the quality of the bond between the materials present at the interface is such that it surpasses the cohesive strength of its individual components and the adhesive strength between the interfacial layers, yielding an adhesive interfacial assembly that exhibits greater strength when acting as a single

body rather than as separate layers. Moreover, the reported fracture modes may only be considered "apparent" as confirmation of "true" failure modes would require the use of sophisticated surface chemistry analysis instead of only high-magnification microscopic evaluation.[39]

Ongoing efforts continue toward gaining a better understanding of the role of MMPs in adhesive interfaces degradation and the anti-proteolytic benefits that may be derived from CHX and other inhibiting agents. Several benefits that may be derived from the use of CHX, namely anti-bacterial, anti-proteolytic, re-wetting, and buffering properties, deserve further investigation. Aspects relative to the specific adhesive approach, pH, and monomeric composition of the adhesive system, as well as the concentration and application protocol for the delivery of CHX, need to be further investigated to be able to maximize its retention and effectiveness while minimizing its potential adverse effects on the polymer network. Furthermore, several other mechanisms that may also contribute to the degradation of adhesive interfaces, namely permeation of dentin fluid esterases into the adhesive interface and proteolytic degradation by cysteine cathepsins, also require further investigation. Most of the available evidence pertaining to the biologic aspects involved in the degradation of adhesive interfaces is derived from laboratory studies. Although *in vitro* studies are effective at isolating the effect of individual variables in the overall process, ultimate validation of the potential therapeutic benefits that may be derived from incorporating inhibiting agents into the adhesive interfaces can only be obtained from clinical studies.

CONCLUSIONS

Within the limitations of this *in vitro* study design, the following can be concluded:

- CHX, in the concentrations of 0.2% and 2.0%, inhibited dentin proteolytic activity as determined by gelatin zymography.
- When CHX was incorporated into a commercially available adhesive or used as a therapeutic primer, no difference in bond strength was observed either at baseline or at six months of storage relative to the control group without CHX irrespective of adhesive approach.

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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