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In Vitro Evaluation of Benzalkonium Chloride in the Preservation of Adhesive Interfaces

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Clinical Relevance

Benzalkonium chloride preserves resin-dentin bonds by reducing collagen solubilization. When incorporated into adhesive blends, benzalkonium chloride provides comparable bond stability to other inhibitors and application protocols without adding more steps to the bonding sequence.

SUMMARY

Inhibition of endogenous dentin matrix metalloproteinases (MMPs) by benzalkonium chloride (BAC) decreases collagen solubilization and may help improve resin-dentin bond stability. Objective: This study evaluated the resin-dentin bond stability of experimental adhesive blends containing BAC and the stability of dentin matrices by assessing the mass loss and collagen solubilization from dentin beams pretreated with BAC.

Materials and Methods: Twenty-five healthy molars were used for the bond strength evaluation of a two-step etch-and-rinse adhesive (Adper Single Bond Plus, SB) modified with BAC or not. The following groups were tested: 1) SB with no inhibitor (control); 2) topical 2.0% chlorhexidine + SB; 3) 1.0% BAC etchant + SB; 4) 0.5% BAC-SB; and 5) 1.0% BAC-SB. Microtensile bond strength (μTBS) and failure mode distribution under standard error of the mean were evaluated after 24 hours and six months of storage in artificial saliva (AS). A two-way analysis of variance and Tukey test with a significance level of $p<0.05$ was used for data analysis. In addition, 30 completely demineralized dentin beams from human molars were either dipped in deionized water (DW, control) or dipped in 0.5% and 1.0% BAC for 60 seconds, and then incubated in AS. Collagen solubilization was assessed by evaluating the dry mass loss and quantifying the amount of hydroxyproline (HYP) released from hydrolyzed specimens after four weeks of incubation.

Results: The control group demonstrated lower μTBS than some of the experimental groups containing BAC at 24 hours and six months ($p<0.05$). When BAC was incorporated into the adhesive blend in concentrations of 0.5% and

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1.0%, no reduction in dentin bond strength was observed after six months ($p<0.05$). Less mass loss and HYP release was seen for dentin matrices pretreated with BAC relative to the control pretreated with DW ($p<0.05$).

Conclusion: This *in vitro* study demonstrates that BAC contributes to the preservation of resin-dentin bonds by reducing collagen degradation.

INTRODUCTION

Resin-dentin bonds created by contemporary dentin adhesive systems still deteriorate over time.[1] The ionic and hydrophilic nature of current adhesives yields permeable hybrid layers that are susceptible to hydrolytic degradation.[1] Collagenolytic activity by host-derived matrix metalloproteinases (MMPs) has also been shown to contribute to the degradation of resin-dentin bonds.[2,3] MMPs, a group of calcium- and zinc-dependent endopeptidases that remain trapped in the dentin matrix during tooth development[4] are known to contribute to the collagenolytic activity in areas of suboptimally infiltrated collagen[3] once they have been activated by exposure to an acidic environment such as the one created by the application of acidic adhesive resins.[5]

Dentin treatment with protease inhibitors has been proposed to prolong the durability of the bonds. Chlorhexidine (CHX), a potent cationic antimicrobial agent[6] and nonspecific dentin MMP inhibitor has been extensively tested for its antiproteolytic effects with good results.[7] However, the large CHX molecule is water soluble and may leach out of the hybrid layer, which limits its long-term antiproteolytic benefits. Another group of antimicrobial agents, the quaternary ammonium compounds (QACs), also display positively charged molecules that bind to negatively charged phosphate and carboxylic groups in hydroxyapatite and collagen respectively. While CHX has two fixed charges, most QACs have only one positive charge.[8] Because QACs are also cationic molecules with antimicrobial properties, it has been speculated that they may display similar anti-MMP properties to CHX, while allowing easier stabilization of the compound within the hybrid layer because of their smaller size. A number of QACs have been recently investigated for their inhibitory properties in dentin MMPs with encouraging results.[8]

Benzalkonium chloride (BAC), a nitrogenous agent containing a quaternary ammonium group, has recently demonstrated effective dentin MMP inhibition.[9] For several years, a phosphoric acid etchant containing 1.0% wt BAC (Etch-37 w/BAC, Bisco Inc, Schaumburg, IL, USA) has been commercially available for its antibacterial properties and has shown no adverse effect on the immediate bond strengths.[10] The additional antiproteolytic benefits that may be derived from the use of BAC have gained attention only recently. Moreover, the issue of the most effective vehicle for the delivery of BAC has also been raised as rinsing the etchant may displace some of the BAC, perhaps limiting the amount that remains viable in the hybrid layer and, thus, its antiproteolytic benefits. A more effective delivery system may incorporate BAC into the primer and/or adhesive formulation. We speculate that incorporating the BAC into the adhesive may yield deeper infiltration of the agent into the demineralized collagen mesh, thus yielding greater antiproteolytic benefits while allowing a simplified clinical application technique.

Therefore, the purpose of this study was to investigate the efficacy of BAC as an inhibitor of dentin MMP activity by quantitatively assessing changes in bond degradation and collagen solubilization. Specific aims included the following: 1) evaluate the resin-dentin bonds created with a commercially available adhesive modified with BAC by means of microtensile bond strength (μTBS) at 24 hours and six months compared with the use of topical 2.0% CHX and BAC-modified phosophoric acid; 2) evaluate the collagen solubilization by assessing the dry mass loss and amount of hydroxyproline released from hydrolyzed specimens after four weeks of incubation dentin matrices pretreated with BAC. The null hypothesis was that dentin treatment with BAC would have no effect on the bond degradation, mass loss and hydroxyproline (HYP) release over time.

MATERIALS AND METHODS

Microtensile Bond Strength (μTBS)

Twenty-five recently extracted, noncarious human molars were used to obtain dentin substrate for bonding. The teeth were obtained under a protocol approved by the State University of New York's Institutional Review Board. A flat, transversely cut surface of superficial/middle dentin was obtained by means of a water-cooled slow-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA), and a smear layer was created with 600-grit silicon carbide abrasive paper (SiC paper, Buehler). The teeth were equally and randomly assigned to five study groups (Table 1) with five teeth in each group as follows: group 1, phosphoric acid treatment followed by

Table 1: *Study Groups, Composition and Application Protocol as per Manufacturer Recommendations*

Group	Code	Description	Adhesive (Composition / Application protocol)
1	Control	PA followed by conventional adhesive	**ADPER SINGLE BOND PLUS (SB**/ 3M ESPE, Lot# 9XT, Saint Paul, MN, USA) Composition (% by wt): Ethyl alcohol (25-35%); silane treated silica nanofiller (10-20%); bisphenol A diglycidyl ether dimethacrylate (BisGMA) (10-20%); 2-Hydroxyethyl methacrylate (HEMA) (5-15%); glycerol 1,3-dimethacrylate (5-10%); copolymer of acrylic and itaconic acids (5-10%); water (<5%); diurethane dimethacrylate (1-5%) Application protocol: • Etch (15 sec). Rinse (10 sec) • Blot dry. Leave surface slightly moist • Apply adhesive (2-3 coats). Scrub (15 sec) • Gently air thin to evaporate solvents (5 sec) • Light cure (10 sec)
2	2.0%CHX	PA followed by 2.0% CHX & conventional adhesive	
3	BAC-PA	1.0% BAC-PA followed by conventional adhesive	
4	0.5% BAC	PA followed by 0.5% BAC-adhesive	
5	1.0% BAC	PA followed by 1.0% BAC-adhesive	

Abbreviations: BAC, benzalkonium chloride; CHX, chlorhexidine; PA, phosphoric acid; SB, adper single bond plus. Conventional adhesive denotes adhesive that was not modified with inhibitor.

conventional adhesive (control); group 2, phosphoric acid treatment followed by topical 2% CHX and conventional adhesive (2.0% CHX); group 3, 1.0% BAC-modified phosphoric acid treatment followed by conventional adhesive (BAC-PA); group 4, phosphoric acid treatment followed by 0.5% BAC-containing adhesive (0.5% BAC); group 5, phosphoric acid treatment followed by 1.0% BAC- containing adhesive (1.0% BAC).

A commercially available aqueous solution of 2.0% CHX digluconate (Consepsis, Ultradent, South Jordan, UT, USA) was used for rewetting dentin in group 2. All groups were treated with 35% phosphoric acid (Ultra-Etch, Ultradent) with exception of group 3, which was etched with phosphoric acid containing 1.0% wt BAC (Etch-37 w/BAC, Lot 1100004919, Bisco). The adhesive used in this study was a two-step etch-and-rinse system (Adper Single Bond Plus, Lot 9XT, 3M ESPE, St Paul, MN, USA). Its composition and application protocol as described by the manufacturer are summarized in Table 1. Benzalkonium chloride was admixed into the primer/adhesive blend in concentrations of 0.5% and 1.0% (wt/vol) for treatment of groups 4 and 5, respectively. The adhesive was applied to the moist dentin surfaces according to the wet-bonding technique 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. Composite build-ups were fabricated with resin composite (Filtek Z100, Lot N372074, 3M ESPE) in shade A2 according to a standardized protocol by applying two increments no greater than 2 mm, each of which was polymerized for 40 seconds.

The restored teeth were stored in distilled water and placed in an incubator at 37°C for 24 hours to ensure adequate polymerization. After 24 hours, all teeth were sectioned, and dentin beams with a cross-sectional area of 0.9 ± 0.1 mm^2 were obtained according to the nontrimming technique.[11] The beams were divided into two equal groups for microtensile bond strength evaluation at 24 hours and after six months of storage at 37°C in an artificial saliva solution containing 0.02% sodium azide. The storage media was replaced with fresh solution once a month to ensure that the preservatives were fresh.

Beams were stressed to failure with a universal testing machine at a crosshead speed of 1 mm/min (Bisco). Failure modes were analyzed by observation by a single examiner (CS) with a stereomicroscope (Nikon SMZ-U, Melville, NY, USA) at a magnification of 50×. The fractured surfaces were classified as follows: 1) cohesive in dentin, 2) adhesive, 3) cohesive in composite, and 4) mixed failure, which was defined as the combination of different failure modes resulting from failure across the interfacial layers.

Because the data were normally distributed (Kolmogorov-Smirnov test), a two-way analysis of variance (ANOVA) was used to analyze the effect of the variables "treatment group" and "storage time" on μTBS. A post-hoc Tukey test was used for pairwise multiple comparisons between group means. A significance level of $p<0.05$ was used for all tests. All statistical analyses were performed with Statistical Package for Social Sciences (SPSS) version 16.0 (SPSS Inc, Chicago, IL, USA).

Collagen Solubilization and Mass Loss

Beam Demineralization and Incubation—Fifteen healthy human molars were obtained under a protocol approved by the State University of New York's Institutional Review Board and stored in a 0.9% NaCl solution containing 0.02% sodium azide at 4°C for no more than three months after extraction. The crowns were separated from the roots at the cementoenamel junction with a slow-speed diamond saw (Isomet, Buehler) and the crowns debrided from enamel, cementum, and pulpal tissue by means of a diamond bur in high-speed handpiece under air-water spray. Midcoronal occusal dentin was exposed by removing the enamel and superficial dentin using a slow-speed diamond saw under water cooling. One dentin disk (1 mm thick) was obtained from each tooth. A total of 30 dentin beams of standardized dimensions ($2.0 \times 1.0 \times 6.0$ mm) were obtained from the dentin disks.

All dentin beams were completely demineralized by immersion in 10 wt% liquid phosphoric acid (pH 1.0, Sigma Aldrich, St Louis, MO, USA) for 18 hours at 25°C under constant stirring, and then rinsed in deionized water (DW) for two hours at 4°C. To determine initial dry mass, the beams were desiccated over anhydrous calcium sulfate for 48 hours and weighed to a constant dry weight in an analytical balance (AG204, Mettler Toledo, LLC, Columbus, OH, USA). After rehydrating the beams in DW for one hour, they were equally and randomly divided in three groups (n=10) and treated with either 0.5% BAC, 1.0% BAC, or DW (control) for 60 seconds, blotted to remove excess, and then incubated in 1 mL of artificial saliva (AS, composed of 12.9 mM KCl, 1.9 mM KSCN, 2.4 mM $Na_2SO_4 \cdot 10\ H_2O$, 3.3 mM NH_4Cl, 1.5 mM $CaCl_2 \cdot 2H_2O$, 7.5 mM $NaHCO_3$, 0.02 mM $ZnCl_2$, and 5 mM HEPES buffer, pH 7.4). The beams were separately incubated in individual screw-capped tubes in a water shaker bath at 37°C. After four weeks, the AS media was analyzed for collagen solubilization by means of hydroxyproline (HYP) release, and the dry mass loss of the beams was determined. To determine dry mass, the beams were dried and weighed as described earlier.

Collagen Solubilization—Quantification of the amount of HYP in the hydrolysates has been proposed as an indirect assessment of collagen degradation as HYP is a relatively unique aminoacid to type I collagen.[12] Its presence in the storage media indicates that collagen peptides from the dentin beams were solubilized over time.[9] An aliquot of 200 μL of the AS solution was mixed with an equal volume of 12 N HCl, and its contents were hydrolyzed to aminoacids in an oil bath at 120°C for 18 hours. The content of each tube was allowed to evaporate to dryness for one week in a large desiccator under a vacuum containing sodium hydroxide and anhydrous calcium sulfate. Hydroxyproline standards were prepared from a 100 μg/mL HYP stock solution in 50% isopropanol. Concentrations of HYP in the standard solution were 0, 5, 10, 15, 20, 25, 30, 35, and 40 μg/mL. Aliquots of 200 μL of each of these standards were hydrolyzed and allowed to evaporate to dryness the same as unknowns. After one week, all residues of dry hydrolysates were evaluated for collagenolytic activity by quantifying the HYP content in the hydrolyzed specimens as per the colorimetric assay by Jamall and others.[13] Briefly, residues of dried hydrolysates, both unknowns and HYP standards, were resolubilized with 1.2 mL of 50% isopropanol and 0.2 mL of chloramine-T solution (1.1 mL of chloramine-T stock solution and 18.9 mL of acetate citrate buffer, pH 6.0). After 10 minutes, 1.0 mL of Ehrlich stock solution (10 g of 4-dimethylaminobenzaldehyde and 11 mL of 60% perchloric acid) in 100% isopropanol was added to give a final volume of 2.4 mL. The specimens were incubated at 50°C for 90 minutes to develop the chromophore. The absorbance values of all specimens' hydrolysates were measured in spectrophotometer (DU 800, Beckman Coulter Inc, Brea, California, USA) at 558 nm against a blank.

A standard curve was created by plotting the absorbance values of the HYP standards against the concentration of HYP in these standards ($R^2 = 0.99$). A regression equation generated by the standard curve was used to calculate the amount of HYP in the unknown samples based on their absorbance values. The resulting amount of HYP, expressed in micrograms, was divided by the original dry mass of each beam yielding the amount of HYP released per milligram of dentin (μg HYP/mg dentin). The data were normally distributed (Kolmogorov-Smirnov test). A one-way ANOVA was used to analyze the "treatment group" effect in both mass loss and HYP release. Post-hoc Student-Newman-Keuls was used for pairwise multiple comparisons between group means. A significance level of $p<0.05$ was used for all tests.

RESULTS

Microtensile Bond Strength (μTBS)

Two-way ANOVA demonstrated a significant effect of the main variables "treatment group" ($p<0.001$) and "time" ($p=0.007$), as well as their interactions

Table 2: *Mean Microtensile Bond Strength (μTBS) Results and Failure Mode Distribution for the Five Study Groups at 24 h and Six mo of Storage (n=10)*[a]

	Control (Group 1)	2.0%CHX (Group 2)	BAC-PA (Group 3)	0.5%BAC (Group 4)	1.0%BAC (Group 5)
24 h μTBS (MPa ± SD)	34.3 ± 7.8 [A,c]	38.3 ± 10.3 [A,b,c]	43.0 ± 11.8 [A,b]	36.4 ± 8.4 [A,b,c]	51.4 ± 7.9 [A,a]
Failure mode A/D/R/M	3 / 0 / 3 / 4	2 / 2 / 1 / 5	2 / 0 / 2 / 6	1 / 2 / 1 / 6	0 / 0 / 2 / 8
6 mo μTBS (MPa ± SD)	27.4 ± 6.2 [B,c]	34.3 ± 5.2 [A,b]	35.1 ± 6.5 [B,b]	36.6 ± 6.2 [A,b]	53.9 ± 6.9 [A,a]
Failure mode A/D/R/M	5 / 1 / 3 / 1	1 / 1/ 3 / 5	2 / 1 / 1 / 6	0 / 1 / 1 / 8	0 / 1 / 2 / 7

Abbreviations: A, adhesive; D, cohesive in dentin; R, cohesive in resin; M, mixed.
[a] *Same superscript letter indicates no significant differences between groups per the results of pairwise multiple comparisons Tukey test (p<0.05). Upper case denotes differences in μTBS values between 24 hours and six months for each of the individual groups (vertical). Lowercase letter denotes differences among treatment groups for each testing time (horizontal).*

(p=0.023) on the bond strength. Table 2 summarizes the mean μTBS values and failure mode distribution for all study groups at 24 hours and six months of storage. At 24 hours, all experimental groups demonstrated higher bond strength than the control group, but only BAC-containing etchant and 1.0% BAC-containing adhesive were significantly higher than the control (p=0.01 and p<0.001 respectively). When bond strength was evaluated at six months, significant differences were observed between the control and all the experimental groups, and the control group showed significantly lower bond strength than all experimental groups (p<0.05). The group treated with 1.0% BAC-containing adhesive demonstrated significantly higher bond strength than all other groups, and the groups treated with 2.0% CHX, BAC-PA, and 0.5% BAC were not significantly different from each other. With exception of the groups treated with BAC-containing adhesive (groups 4 and 5), all groups demonstrated a decrease in bond strength after six months of storage. This decrease was significant for the control group and the group treated with BAC-containing etchant (p<0.05) but not for the group treated with 2.0% CHX. The most prevalent failure modes observed were adhesive and mixed.

Collagen Solubilization and Mass Loss

When the MMP inhibitory properties of BAC were evaluated by quantifying the amount of HYP present in the AS incubation media after four weeks, a significant effect of the "treatment group" (p<0.001) was demonstrated. After four weeks of incubation, the storage media derived from the beams pretreated with 0.5% BAC and 1.0% BAC demonstrated significant less release of HYP than the media derived from the control beams pretreated with DW (p=0.003 and p<0.001, respectively). The amount of HYP in the media from beams pretreated with either 0.5% BAC or 1.0% BAC was not significantly different from each other (Figure 1).

Evaluating BAC MMP inhibitory properties by assessing the dry mass loss from dentin beams after four weeks of incubation also yielded a significant effect of the treatment group (p=0.018). After four weeks, the loss of dry mass was 15.7%, 11.8%, and 6.8% for beams pretreated with DW, 0.5% BAC, and 1.0% BAC, respectively. Only the beams pretreated with 1.0% BAC yielded significantly less mass loss than the control group (p=0.014). No significant differences were seen between beams pretreated with DW and 0.5% BAC, or 0.5% BAC and 1.0% BAC (Figure 2).

DISCUSSION

The present study evaluated changes in bond degradation and collagen solubilization from dentin matrices treated with BAC as an indirect assessment of its efficacy as an inhibitor of dentin MMP activity.

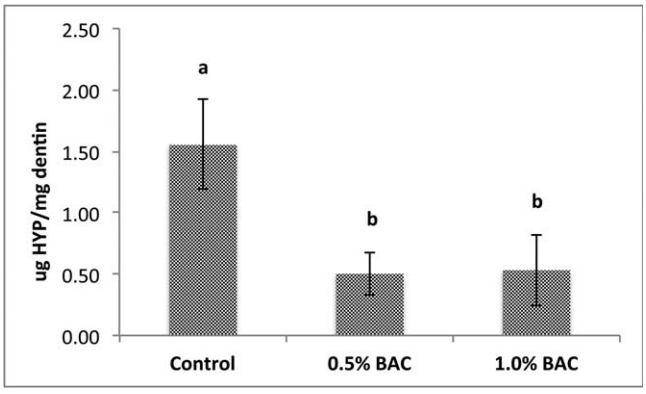

Figure 1. *HYP release from dentin collagen matrices after four weeks of incubation in AS (n=10). Control beams were pretreated with DW for 60 seconds and then incubated in AS. Experimental beams were pretreated with BAC in either 0.5% or 1.0% for 60 seconds. and then incubated in AS. Bars represent the mean values; brackets indicate the standard deviation values. Groups identified by different letters are significantly different (Student-Newman Keuls;* p < *0.05).*

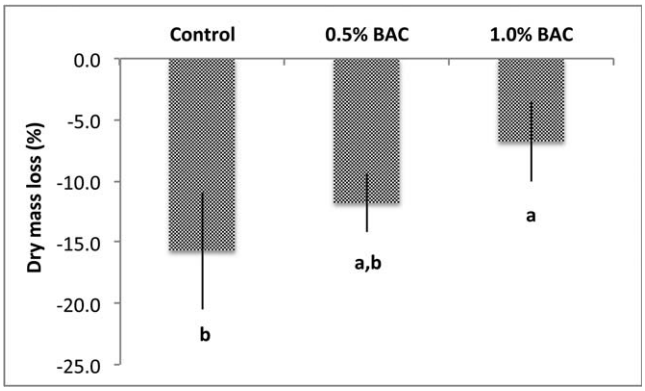

Figure 2. *Percent total dry mass loss of completely demineralized human dentin beams after four weeks of incubation in AS (n=10). Control beams were pretreated with DW for 60 seconds and then incubated in AS. Experimental beams were pretreated with BAC in either 0.5% or 1.0% for 60 seconds and then incubated in AS. Bars represent the mean values; brackets indicate the standard deviation values. Groups identified by different letters are significantly different (Student-Newman-Keuls;* $p < 0.05$*).*

The null hypothesis was rejected as BAC demonstrated both decreased bond degradation after six months and less collagen solubilization after four weeks of incubation. Our results confirm the previously demonstrated effectiveness of BAC as an MMP inhibitor.[9] Incorporatimg BAC, in concentrations of 0.5% and 1.0%, into a commercially available adhesive blend, Single Bond, yielded no bond degradation after six months of storage in AS. A decrease in bond strength after six months was demonstrated for all other groups, which was significant for the control group with no inhibitor and the group treated with BAC-containing etchant, but not for the group treated with 2.0% CHX. Although our results showed stability of the resin-dentin bonds after six months with the use of a BAC-modified adhesive, it is possible that this positive effect may have been the result of an incubation time of only six months; longer incubation periods may yield different results after BAC is allowed to leach out of the hybrid layer. Nonpolymerizable MMP inhibitors such as CHX and BAC are known to bind to dentin electrostatically.[14] Noncovalently bound molecules may leach out of the hybrid layer, compromising its long-term antiproteolytic benefits, and consequently only delaying but not preventing the degradation of adhesive interfaces.[15] Because of the weak electrostatic bonds between BAC and dentin, longer incubation periods may be necessary to evaluate its long-term antiproteolytic benefits. Our results confirm the findings of previous studies, which have shown that BAC used in combination with etch-and-rinse adhesives had no effect on the immediate bond strength.[16-18] These findings suggest that the antimicrobial can be safely combined with the resin monomers present in Single Bond without a compromise to its initial bond strength. Moreover, increased bond strength values were seen for BAC-treated groups relative to the control; the effect was shown to be dose-dependent, with mean bond strength values of 36.4 and 51.4 MPa for 0.5% and 1.0% BAC, respectively, at 24 hours, and 36.6 and 53.9 MPa for 0.5% and 1.0% BAC, respectively, at six months.

BAC, in concentrations of 0.5% and 1.0%, was also evaluated by colorimetric assay to determine collagen solubilization. In this model, demineralized dentin beams were pretreated with 0.5% or 10% BAC to allow the BAC to diffuse into the water-filled spaces between the collagen fibrils and within the dentinal tubules for 60 seconds, and then were incubated in BAC-free AS media. Significantly less release of HYP was seen when dentin beams were pretreated with 0.5% and 1.0% BAC. This finding confirms the results of a recent study that showed that BAC in concentrations of 0.5% or greater can inhibit MMPs.[9] Evaluation of the dry mass loss of the dentin beams after pretreatment with DW, 0.5% BAC, or 1.0% BAC also revealed differences; beams pretreated with 1.0% BAC showed significantly less mass loss than the beams pretreated with DW (control). There were no detectable differences between 0.5% and 1.0% BAC for HYP release or mass loss. Less solubilization of collagen is expected when BAC binds to collagen and MMPs bind to collagen as these molecular interactions are known to dissociate the enzyme's tertiary structure.[19] Nevertheless, a recent study investigating the binding ability of BAC to demineralized dentin found that not all the BAC binds to collagen. The BAC that is not bound but remains trapped in the water of the interfibrilar spaces can be easily removed by water rinsing as evidenced in that study by the loss of 50% of the BAC after water rinse.[9] Clinically, this indicates that phosphoric acid etchant may not be the most effective vehicle for the delivery of BAC. A significant 18.2% decrease in bond strength after six months when BAC was delivered into the etchant confirms this notion.

Protease inhibitors are commonly delivered either topically before the application of the adhesive or in conjunction with the phosphoric acid etchant. This, aside from introducing an additional step to the bonding sequence, may compromise the amount of agent that remains viable in the hybrid layer after rinsing the etchant. Attempts to incorporate

these agents into primers/adhesive blends have shown encouraging findings, and our results support this concept. Reduction in bond degradation has been observed when CHX was incorporated into the primer of two-step self-etch adhesives.[20,21] To date, no studies have evaluated the stability of adhesive interfaces after treatment with adhesive blends modified with BAC, and thus a direct comparison of our results with those from other studies is not possible. We can speculate, based on our results, that treatment of the acid-etched dentin with a therapeutic primer/adhesive blend containing BAC may allow greater diffusion of the agent into the water-filled spaces between the collagen fibers and dentinal tubules. We can further speculate that incorporating BAC into the primer/adhesive blend may prolong its availability within the hybrid layer with the assumption that the resin matrix may act as a reservoir for its slow release over time. BAC is known to be soluble in both ethanol and acetone, and its activity not greatly affected by pH, which may suggest that its antiproteolytic benefits may be safely extrapolated to adhesives of different composition. However, no assumptions can be made, and studies should be conducted to understand specific interactions between BAC and adhesives of different acidity, hydrophilicity, and monomeric composition as well as to determine its effect on the properties of the polymerized resin matrix.

Efforts continue toward gaining a better understanding of the many aspects that play a role in the long-term success of adhesive restorations. Other approaches also known to reduce collagen degradation and preserve dentin-resin interfaces are being investigated. Increasing the extent of collagen cross-linking before adhesive application,[22] and use of polymerizable acrylate or methacrylate groups, which can be stabilized within the hybrid layer, are examples of these. These interventions, however, still fail to address the critical issue of the water that remains entrapped within the collagen intrafibrilar compartments, weakening the interface and providing the functional medium for MMP activity. In this regard, molecular immobilization of MMPs through remineralization of water-rich collagen fibrils within the hybrid layer may represent a more permanent strategy to prevent organic matrix degradation.[23] However, despite the many aspects involved in the degradation of adhesive interfaces, our study clearly shows the importance of dentin MMP inhibition in the preservation of hybrid layers over time.

CONCLUSIONS

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

- An improved stability of the resin-dentin bonds after six months was demonstrated with the use of BAC-containing adhesives relative to the control group with no inhibitor.
- Beam treatment with 0.5% BAC and 1.0% BAC for 60 seconds yielded significantly less HYP release from dentin matrices, as determined by colorimetric assay indicating that BAC prevents collagen solubilization. This effect was shown to be dose-dependent.
- Beam treatment with 0.5% BAC and 1.0% BAC for 60 seconds yielded significantly less dry mass loss after four weeks, indicating that BAC prevents collagen solubilization.

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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