Dentin Bonding: Matrix Metalloproteinases and Chlorhexidine

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Contemporary resin–dentin bonding is initiated by systems that use phosphoric acid or acidic resin monomers to remove mineral, exposing the superficial dentin collagen matrix. Collagen-associated proteins, including enzymes known as matrix metalloproteinases (MMPs), also are exposed. The collagen matrix is subsequently infiltrated with resins that are polymerized to establish an adhesive attachment to the dentin. Exposed collagen matrix that is not infiltrated with the adhesive can be degraded by associated MMPs, which might result in deterioration of the adhesive–dentin bond over time. Chlorhexidine (CHX) is able to inhibit MMPs by binding calcium and zinc ions necessary for proteolytic activity. This Critical Appraisal presents salient publications on research that evaluate CHX and its ability to limit MMP degradation of dentin bonds created by etch-and-rinse and self-etch adhesive systems.

In Vivo Preservation of the Hybrid Layer by Chlorhexidine

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ABSTRACT

Objective: This study evaluated whether chlorhexidine (CHX) would interfere with dentin bonding, as well as its ability to prevent or delay degradation of the resin–dentin bond in human third molars in vivo. An ethanol-based bonding system was used as part of a two-step total etch-and-rinse procedure for Class I composite restorations.

Materials and Methods: Twelve subjects, each with a pair of non-carious erupted third molars scheduled for extraction, were enrolled in this study. Class I preparations were created in the molars. These were restored using a 15-second etch with 35% phosphoric acid, bonding with Single Bond (3M ESPE, St. Paul, MN, USA), a microfilled composite liner, and a highly filled composite resin (Z250, 3M ESPE). One of the two molars in each subject was treated with a 60-second application of 2 wt% CHX solution (PRODERMA, Piracicaba, SP, Brazil) to the acid-etched dentin. All restorations were placed under rubber dam isolation and had continuous cavosurface margins in enamel. A moist-bonding technique was used.

Three pairs of teeth (experimental and control) were immediately extracted and subjected to analysis. The other nine pairs were examined periodically and extracted 14 months later. Once extracted, the teeth were sectioned and microtensile bond strengths were determined. Transmission electron microscopy (TEM) was used to evaluate the failure mode of specimens subjected to bond strength testing as well as the

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morphologic characteristics of the hybrid layer of the various intact specimens.

Results: All teeth were asymptomatic and the restorations showed no clinical signs of failure. There was no statistically significant difference in the immediate bond strengths of the CHX-treated teeth and the controls. After 14 months in vivo, the bond strengths of the CHX-treated teeth remained the same, whereas the bond strengths of the control teeth decreased by a statistically significant 38%.

TEM revealed that both the CHX-treated and control bonds failed primarily by mixed fractures. CHX-treated specimens had a lower failure rate within the hybrid layer than control teeth. Evaluation of intact specimens revealed that the hybrid layers of the CHX-treated and control (immediately extracted) as well as the CHX-treated specimens (extracted after 14 months) were all intact. This was in contrast to the hybrid layers of the control specimens extracted after 14 months, which revealed various zones of disintegration and evidence of collagen fibril degradation.

Conclusions: The treatment of acid-etched dentin with CHX did not interfere with the ability of an ethanol-based adhesive to initially form a dentin hybrid layer. In addition, hybrid layers formed in the presence of CHX, a known MMP inhibitor, did not reveal degradation of collagen fibrils associated with the hybrid layer after 14 months in vivo.

Summary: This paper describes how the bond created with an ethanol-based, two-step etch-and-rinse adhesive can be stabilized for up to 14 months in vivo by treatment of acid-etched dentin with 2% CHX for 60 seconds. This lends further support to the notion that collagen-associated MMPs exposed during acid-etching can degrade exposed collagen, resulting in fairly rapid breakdown of the dentin–adhesive interface.

COMMENTARY

This is excellent, breakthrough clinical research by a team of expert researchers representing Brazil, Finland, Italy, and the United States. In addition to negative influences of water or oral/dentinal fluids on composite resins, clinicians and researchers alike should understand the potential influence of non-collagenous proteins present in dentin and the conditions under which these proteins interact with the exposed collagen fibrils immediately beneath the hybrid layer as well as fibrils that become exposed over time as adhesives undergo water sorption.

The authors point out that this research does not prove that an inhibitory effect of CHX on host MMPs was the only reason for preservation of bond strengths and hybrid layer morphology in vivo. However, it stands to reason that the inclusion of CHX in clinical protocols that use etch-and-rinse adhesive systems might increase the predictability of the dentin bond, at least for the short term. The presence of CHX does not eliminate the negative results of hybrid layer water sorption ultimately leading to bond deterioration. These results were found in restored Class I preparations and might not apply to Class II preparations with cavosurface margins that are not bonded to enamel.

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In Vivo Chlorhexidine Stabilization of Hybrid Layers of an Acetone-Based Dentin Adhesive

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ABSTRACT

Objective: This study evaluated whether CHX would prevent degradation of the hybrid layer formed in the deep dentin of premolars after 12-months clinical service in vivo. An acetone-based bonding system was used as part of a two-step etch-and-rinse procedure for Class I composite restorations.

Materials and Methods: Eight pairs of non-carious premolars scheduled for extraction, in six subjects, were used in this study. Moderately deep Class I preparations (3 mm) were made in the teeth and were restored using a 15-second etch with 34% phosphoric acid, bonding with Prime & Bond NT (Dentsply/Caulk, Milford, DE, USA), a 1-mm thick microfilled composite layer, and a microhybrid composite resin (TPH, Dentsply/Caulk). One of the two bilateral premolars in each subject was treated with a 30-second application of 2 wt% CHX solution (Cavity Cleanser, BISCO, Itasca, IL, USA) to the acid-etched dentin. All restorations were placed under rubber dam and had continuous cavosurface margins in enamel. A moist bonding technique and incremental composite placement technique were used.

The restored teeth were extracted 12 months later. Once extracted, experimental and control specimens were sectioned and the morphologic characteristics of the hybrid layers on the pulpal floors were evaluated using TEM.

In vitro microtensile bond strength testing was accomplished on 10 other, non-subject-related, teeth. Class I preparations (3 mm deep) were made in the teeth and were restored using the same methods used clinically.

Results: All teeth were asymptomatic and the restorations showed no clinical signs of failure when they were extracted. TEM evaluation revealed that the hybrid layers of the CHX-treated teeth were all intact and demonstrated preservation of the collagen fibrils in the hybrid layers. This was in contrast with the control specimens, which had extensive destruction of the hybrid layer, including loss of the collagen fibrils at 12 months after restoration.

The microtensile bond strength revealed no statistically significant difference in the immediate bond strengths of CHX-treated specimens and the controls.

Conclusions: Treatment of acid-etched dentin with CHX did not interfere with the ability of an acetone-based etch-and-rinse adhesive to initially form a dentin hybrid layer. After 12 months in vivo, hybrid layers formed using the same system had no evident degradation of hybrid layers formed in the presence of CHX.

Summary: This paper describes how, when using an acetone-based adhesive system, a 30-second application of CHX prevented disintegration of the hybrid layer collagen fibrils for up to 12 months in vivo. Additional in vitro testing indicated that CHX did not compromise the potential strength of the adhesive bond to dentin. As with the previous paper, this is evidence that collagen-associated MMPs exposed during acid-etching can degrade exposed collagen.

COMMENTARY

This report, by a team of dental researchers representing the United States, Mexico, and China, provides useful insight into the potential clinical performance of hybrid layers created using an acetone-based adhesive system. These results suggest a potential benefit in the use of CHX immediately after acid-etching when using such adhesive systems. The authors point out that this research was done under ideal conditions that excluded the potential hydrolytic influences of contamination with saliva as well as the collagenolytic influence of salivary MMPs. Less-than-ideal clinical conditions might limit the

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potential effectiveness of CHX use. As in the case with the previous study, these relatively short-term results were found in Class I restorations and might not apply to proximal preparations with cavosurface margins that are not bonded to enamel. Some have expressed concern that CHX associated with the exposed collagen matrix in the hybrid layer might be gradually lost through water sorption. Additional calcium and zinc cations, made available through the dentinal tubules, might result in MMP reactivation. MMP-induced hybrid layer disintegration could then continue.

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Inhibition of Enzymatic Degradation of Adhesive–Dentin Interfaces

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ABSTRACT

Objective: This in vitro study evaluated whether CHX (a *non-specific* MMP inhibitor) and SB-3CT (a *specific* MMP-2/MMP-9 inhibitor not suitable for human use) would prevent degradation of the hybrid layer formed when using three-step etch-and-rinse (with an ethanol-based primer) and two-step self-etch adhesive systems. The presence of dentin MMP activity and the influence of adhesive primers on activated MMP-2 was assessed by gelatin zymography. Hybrid layer degradation was assessed by microtensile bond strength testing and TEM.

Materials and Methods: Human dentin powder, prepared from extracted third molars, was exposed to the primer from a three-step etch-and-rinse (OptiBond FL, SDS-Kerr, Orange, CA, USA) or a two-step self-etch (Clearfil SE Bond, Kuraray, Osaka, Japan) adhesive system using manufacturer-recommended primer contact times but without light activation. In addition, dentin powder was exposed to 35% phosphoric acid alone. Untreated dentin powder was used as a control. Acetone was used to remove the resin materials from the dentin powder after exposure to the primers. The relative levels of extracted and activated gelatinases (MMP-2, MMP-9) in the dentin powder supernatants were assessed by gelatin zymography.

Composite resin (Clearfil AP-X, Kuraray) was bonded to human mid-coronal intact dentin surfaces using the two adhesive systems according to their manufacturers' instructions. Additional dentin surfaces were bonded using the two adhesive systems with primers modified by the addition of 0.05% CHX (chlorhexidine diacetate powder, FlukaSigma-Aldrich, St. Louis, MO, USA) or 10 μM SB-3CT (Alexis Biochemicals, Lausen, Switzerland). The restored teeth where sectioned after water storage (1 week; 3, 6, or 12 months) and the microtensile bond strengths of the adhesive interfaces were measured. The morphologic characteristics of the hybrid layers of samples from each group were evaluated using TEM.

Results: Gelatin zymography revealed that treatment of the dentin powder with phosphoric acid alone or as part of the three-step etch-and-rinse procedure resulted in increased MMP-2 gelatinolytic activity over controls. OptiBond FL primer did not increase MMP-2 activity over that of the phosphoric acid alone. Treatment of the dentin powder with the Clearfil self-etch primer did not result in any detectable gelatinolytic activity.

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Addition of phosphoric acid or adhesive primers to a solution of activated MMP-2 (no dentin powder) resulted in the elimination of the MMP-2 gelatinolytic activity.

There was a statistically significant decrease in the microtensile bond strengths of both adhesive systems by 12 months. The bond strengths of the three-step etch-and-rinse adhesive declined more rapidly and to a greater extent than the two-step self-etch adhesive. Addition of CHX or SB-3CT to the primers did not compromise immediate bond strengths, but did not counteract the decline in bond strengths after 12-months water storage.

TEM analysis of specimens from both adhesive systems revealed mixed adhesive–cohesive failures with progression toward primarily adhesive interface failures at 12 months. Hybrid layer degradation over time was not extensive for either adhesive system, and addition of MMP inhibitors did not modify the ultrastructural appearance of the hybrid layers.

Conclusions: Protein extracts from dentin powder that were treated with phosphoric acid alone or as part of a three-step etch-and-rinse procedure demonstrated increased in vitro MMP activity. Protein extracts from dentin powder that had been treated with the acidic primer component of the two-step self-etch system did not demonstrate increase in vitro MMP activity. The inclusion of 0.05% CHX as a component of the primers did not interfere with dentin bonding, but it also did not prevent a reduction in bond strengths over time in the laboratory setting. The morphologic characteristics of the hybrid layer formed by the two adhesive systems at baseline and at 12-months water storage were similar.

Summary: This research demonstrated that active MMPs are released from dentin powder when it is etched with phosphoric acid. Data presented indicated that a three-step, etch-and-rinse adhesive system demineralized dentin particles enough to release detectable MMPs whereas a two-step, self-etch adhesive system did not demineralize dentin particles enough to detect MMP activity. Microtensile bond strength testing was used to evaluate the stability of adhesive bonds to intact dentin. Evaluation of bonds created with the three-step etch-and-rinse and the two-step self-etch systems revealed that CHX did not interfere with initial bond formation and did not limit bond strength deterioration after 12-months storage in water. Transmission electron microscopy revealed hybrid layers created with these adhesive systems appeared the same after laboratory storage in water for 12 months, whether CHX was used or not.

COMMENTARY

This research was accomplished by a well-respected group of experts in adhesive dentistry from Belgium. Consideration of this in vitro study and the in vivo studies presented above provides a reasonable level of assurance that CHX does not interfere with the establishment of a resin–dentin hybrid layer when using three-step etch-and-rinse or two-step self-etch adhesive systems.

Comparison of these studies highlights the differences between bonding to a live tooth and an extracted tooth. A vital tooth has a pulpal response (via the dentinal tubules) and is subjected to intermittent thermal cycling and cyclic loading as well as other factors in the oral environment. The MMP response of a vital dentin–pulp complex is not reproducible in the dental laboratory.

The two-step self-etch primer system used in this study is considered to be only mildly acidic (causing limited demineralization of the dentin), resulting in no detection of active MMPs under the conditions used in the study. However, there was a reduction in bond strengths over time with or without the use of CHX. This lends support to the notion that a gradual hydrolysis of the bonding resin in the hybrid layer occurs over time. Self-etch systems with more acidic resin primers might result in greater activation of MMPs, causing degradation of the collagen in the hybrid layer.

There are currently no studies evaluating the one-step self-etch adhesive systems and the influence of CHX on short-term or long-term stabilization of the hybrid

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layer. As with other types of adhesive systems, the in vitro bond strengths of these systems decrease over time.

In general, bond deterioration can occur beneath the hybrid layer, within the hybrid layer, and at the interface between the adhesive and the restorative material. Degradation of exposed collagen beneath or as part of the hybrid layer is a result of MMP collagenolytic activity. Degradation of the adhesive–resin components occur through water sorption leading to hydrolysis and chemical degradation. Systems that provide optimal resin infiltration of the exposed collagen matrix could result in more stable long-term adhesive bonds.

It is important to note that this Critical Appraisal is focused on the potential use of CHX to limit the negative influence of host MMPs on the hybrid layer formed during dentin bonding. The relative clinical significance of these findings should be considered in light of the overall satisfactory clinical performance of composite resin restorations in contemporary dental practice. However, because concerns remain with regard to the increased risk of secondary caries associated with large Class II composite restorations, ongoing research seeking to improve the predictability of the dentin bond is indicated.

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THE BOTTOM LINE

- Phosphoric acid-etching of dentin results in exposure of superficial collagen and collagen-associated MMPs. Activation of the MMPs with subsequent degradation of the exposed collagen results in gradual deterioration of the hybrid layer.
- Treatment of acid-etched dentin, whether shallow or deep, with a 30- to 60-second application of a 2% CHX solution does not appear to compromise the formation of the hybrid layer formed with current dentin adhesive systems.
- Chlorhexidine digluconate, a known MMP inhibitor, appears to help clinically stabilize the hybrid layer on the pulpal floor of Class I restorations up to 14 months when using ethanol- or acetone-based etch-and-rinse adhesive systems.
- Evidence for the bond-stabilizing influence of CHX is based on short-term clinical and laboratory studies. It remains to be seen whether CHX has a long-term effect on durability of resin–dentin bonds.

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