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

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Clinical Relevance

Extensive degradation of dentin hybrid layers formed with an acetone-based dentin adhesive beneath Class I resin composite restorations was evident after one year unless the teeth received an application of 2% chlorhexidine digluconate after etching.

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SUMMARY

The current *in vivo* study evaluated the degradation of dentin hybrid layers in deep occlusal-surface resin composite restorations using TEM. Caries-free premolars scheduled for extraction as part of orthodontic treatment were prepared and restored, then extracted after 12 months. The adhesive used was a single-bottle etch-and-rinse acetone-based product (Prime & Bond NT, Dentsply/Caulk). Control group restorations (n=8) were placed according to the manufacturer's instructions, while the experimental group received application of a 2% solution of chlorhexidine digluconate after etching and rinsing and prior to application of the adhesive. Extensive degradation was observed in all of the teeth in the control group after 12 months, while no degradation was observed in the experimental group. *In vitro* testing showed no significant difference in immediate microtensile bond strength between the control and experimental adhesive protocols.

INTRODUCTION

The adhesion of resin to dentin occurs through infiltration into and polymerization of hydrophilic resins within the collagen mesh exposed through acid decalcification of dentin, forming a hybrid layer.[1] The stability of the hybrid layers has been investigated in three clinical studies of 6-12 months' duration, in which resin restorations placed in teeth destined for extraction have been evaluated after extraction via electron microscopy for degradation of the underlying hybrid layers.[2-4] Unfortunately, all of these studies have demonstrated degradation of the hybrid layers beneath resins placed with standard adhesive procedures, especially after one year. This is probably due to hydrolysis of collagen[5] produced by metalloproteinase (MMP) enzymes found within dentin.[6] Although dentin does not remodel, these enzymes are present in all collagen and contribute to the progression of caries through removal of the dentinal collagen exposed by demineralization.[7-8] As in the carious process, these enzymes become activated during adhesive procedures when dentin is acid-etched and can slowly degrade resin-dentin interfaces over time.[5-6]

It is known that chlorhexidine is an inhibitor of MMP activity,[9-10] so each of the above-cited clinical studies included an experimental group in which a 2% solution of chlorhexidine digluconate was applied to the dentin after acid etching and rinsing and just prior to bonding. Teeth from these groups showed no degradation of the hybrid layers. One limitation of the extant literature is that all of the clinical studies in this area have employed the same single-bottle, alcohol-based adhesive (Adper Single Bond Plus, 3M ESPE Dental Products, St Paul, MN, USA),[2-4] so it is not known with certainty whether these results will generalize to other classes of adhesive.

The current study applied the protocol using premolars from Brackett and others[4] to restorations placed with an acetone-based adhesive to determine whether the apparent rate of degradation and the effect of chlorhexidine application is the same over 12 months as has been observed with an alcohol-based adhesive. The effect of chlorhexidine application on immediate *in vitro* dentin bond strength of the same adhesive used was also evaluated.

METHODS AND MATERIALS

Clinical Procedures

All clinical procedures were carried out at the dental school clinic of the Facultad de Estomatologia, Benémerita Universidad Autónoma de Puebla. The current study was conducted in accordance with all local regulations for the ethical treatment of human subjects.

Eight pairs of like contralateral caries-free premolars, which had been scheduled for extraction as part of orthodontic treatment, were identified in six healthy subjects with a median age of 14 years. Each received an occlusal cavity preparation of approximately 3 mm in pulpal depth and 2.5 mm in width. The preparations were performed with a new 245 carbide bur (Brasseler USA, Savannah, GA, USA) at high speed under local anesthesia, using rubber dam isolation and liberal air-water coolant. A member of each pair of teeth was assigned randomly to either the control or experimental group.

Restorations in the control group were preceded by 15 seconds' etching of preparations with 34% phosphoric acid gel (Caulk Tooth Conditioner Gel, Dentsply/Caulk, Milford, DE, USA). After rinsing and brief air drying, a single-bottle adhesive (Prime & Bond NT, Dentsply/Caulk) was applied three times, with agitation of the adhesive for 15 seconds, followed by thorough air drying and light curing for 10 seconds. To facilitate future preparation of microscopic specimens, an approximately 1-mm thick layer of microfilled resin composite (Epic TMTP, Parkell, Inc, Edgewood, NY, USA) was placed on the pulpal floor and light cured for 20 seconds. The restorations were completed with a microhybrid resin composite (TPH[3] [Dentsply/Caulk]), which was placed in increments under 2 mm in thickness, each of which was light cured for 40 seconds. Light output of the curing light used (Model SL3000, 3M ESPE) was found to exceed 450 mW/cm^2 prior to and after the study and was verified during placement of the restorations using the unit's built-in radiometer. Finishing was accomplished using carbide finishing burs (ET, Brasseler) and rubber polishing points (Enhance, Dentsply/Caulk).

Restoration of the teeth in the experimental group followed the same protocol as above except that, following etching, rinsing and removal of excess water, the acid-etched preparation was treated with 2% chlorhexidine digluconate (Cavity Cleanser, BISCO, Inc, Schaumburg, IL, USA), using a foam pellet saturated with the solution for 30 seconds. Excess solution was removed with a new dry pellet, leaving the dentin surface visibly moist. All the teeth were prepared and restored by the same operator.

After 12 months, the restored teeth were extracted under local anesthesia. After extraction, the apical two-thirds of the roots were removed with a high-speed bur with water coolant to facilitate penetration of fixative. The remaining crown/root segments were placed into Karnovsky's fixative (2% paraformaldehyde and 2.5% glutaraldehyde in a 0.1 M phosphate buffer titrated to pH 7.4) for seven days, then processed for transmission electron microscopy.

Laboratory Procedures

All laboratory procedures were performed at the Medical College of Georgia, School of Dentistry, follow-

ing that institution's regulations for the ethical treatment of human subjects.

When the specimens were received, each tooth was sectioned faciolingually into four 1-mm thick serial sections, each containing a part of the restoration. Two sections from each tooth were randomly selected and completely demineralized in ethylene diamine tetraacetic acid. Demineralized epoxy resin-embedded 90-mm thick sections were prepared according to the TEM protocol of Tay and others,[11] which entails staining with 2% uranyl acetate and Reynolds' lead citrate for examination of the characteristics of the resin-dentin interfaces, and with 1% phosphotungstic acid and 2% uranyl acetate for examination of the status of the collagen fibrils. The sections were examined in a TEM (Philips EM208S, Eindhoven, The Netherlands) operated at 80 kV. Resin-dentin interfaces from the pulpal floors of the control and the experimental teeth were examined.

Immediate microtensile bond strengths to dentin were determined on extracted teeth not derived from the subjects in the clinical portion of the study in order to ensure that the use of chlorhexidine did not impair the adhesion of resin to dentin. The same adhesive protocols and materials listed above were used, except that no layer of microfilled resin was placed in order to avoid the use of two resin composites of differing elastic modulus, which could compromise the bond strength data. A non-trimming method previously described was employed.[12] The sample size was five teeth/35 beams; the teeth were sectioned one day after restoration and the beams were tested one day after sectioning. The type of failure was visually categorized at 2.5x magnification as adhesive, cohesive or mixed. Because multiple beams derived from the same tooth are not independent samples, bond strengths were calculated for each beam, then the averages of the beams calculated for each tooth were used as the five samples per group. Bond strength was calculated, and data obtained for the two groups was statistically analyzed using a two-sample *t*-test at a significance level of 5%.

RESULTS

Microtensile dentin bond strengths from both the chlorhexidine-treated and control groups of specimens were found to be normally distributed, and no significant difference was found between the two ($p>0.99$). The means (SD) were 55.0 (13.6) and 55.4 (11.2) MPa, respectively, and all failures were classified as adhesive.

No patient complained of post-operative sensitivity, and all the restorations were clinically serviceable until the date of extraction. No evidence of degradation was observed in the hybrid layers of any restoration in the experimental group (Figure 1), but extensive deterioration of the hybrid layers, manifesting as loss of cross-banding of collagen fibrils, was evident in all eight teeth comprising the control group (Figure 2). Remnants of banded collagen and dentinal tubules in the control specimens demonstrated that electron-lucent areas did result from collagen degradation and not from errors in adhesive technique (Figure 3).

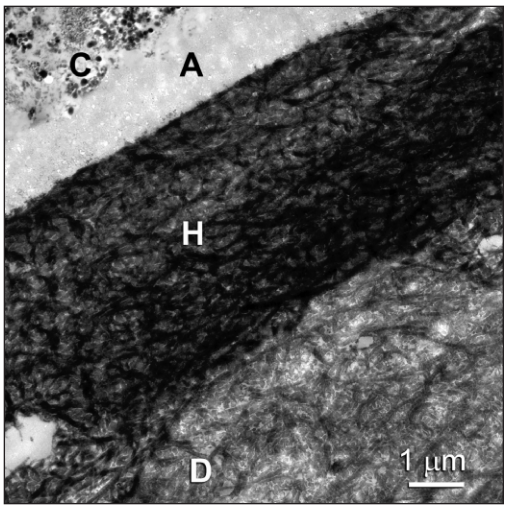

Figure 1. *Representative TEM of the resin/dentin interface of teeth in the experimental group after 12 months* in vivo*, demonstrating preservation of cross-banded collagen fibrils within the hybrid layer (H). (C=microfilled resin composite; A=adhesive layer; D=mineralized dentin; original magnification = 7500x).*

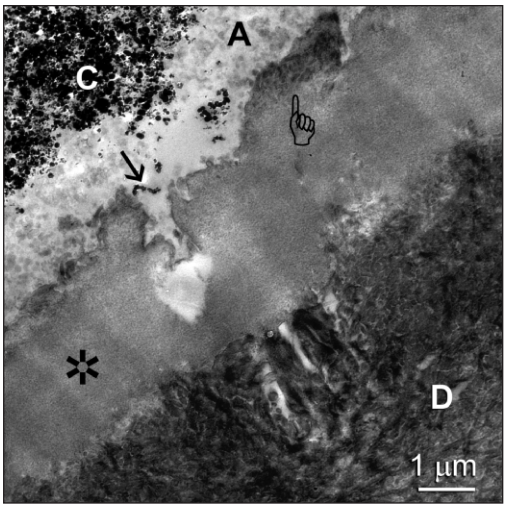

Figure 2. *Representative TEM of the resin/dentin interface of teeth in the control group, which demonstrated significant degradation of hybrid layers after 12 months* in vivo*. Loss of collagen is evident as the electron-lucent zone replacing almost the entire hybrid layer (asterisk). Resin infiltration into the site of dentin tubule is evident (arrow), as is residual banded collagen near the adhesive layer (index finger). (C=microfilled resin composite; A=adhesive layer; D=mineralized dentin; original magnification = 7500x).*

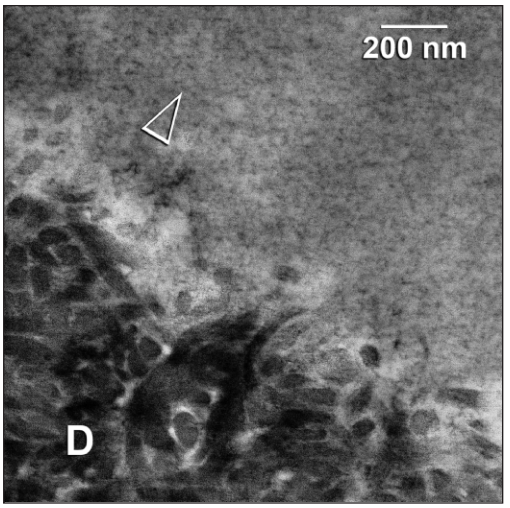

Figure 3. *Representative TEM of the resin/dentin interface of teeth in the control group after 12 months* in vivo *at higher magnification, demonstrating cross-banding of the collagen fibrils in the mineralized dentin layer (D) but loss of banded collagen within the hybrid layer (arrow). Transition between these zones demonstrates the hybrid layer to originally have been made up of banded collagen and resin. (Original magnification = 25,000x).*

DISCUSSION

Since resin composite restorations are known to have a far greater service life than the apparent one-year lifespan of hybrid layers demonstrated in this and other studies, it is likely that enamel adhesion largely preserves the integrity of resin restorations past the first year of service. The use of chlorhexidine solution after etching, as described here, does not appear to have detrimental effects either on *in vitro* dentin bond strength of the adhesive employed or on the clinical serviceability of Class I resin composite restorations over 12 months. However, the authors urge that clinicians should be cautious in adopting the use of chlorhexidine after etching, as clinical data beyond one year and for other types of restoration than Class I are not available.

Although it is a subjective judgment, it appears that degradation of the hybrid layers produced with an acetone-based adhesive in the current study proceeded more rapidly than the degradation of hybrid layers in a similar-duration study done with an alcohol-based adhesive.[2] Possibly, the latter infiltrated into the collagen fibrils better or it had a greater inhibitory effect on dentinal MMP enzymes, which, in the opinion of the investigators, was the most likely cause of the degradation observed in the control group. This is probable, since the teeth restored could be ideally isolated and near-perfect enamel margins produced and because no degradation was evident in the experimental group, which had undergone treatment with an MMP inhibitor.

Preparations in the current study were deliberately extended into dentin near the pulp, in which MMPs are more prevalent,[8] to simulate the degradation of hybrid layers in carious teeth, which would be promoted by dentinal MMPs already activated by caries. The ideal circumstances under which restorations could be placed in the current study, in which saliva was completely excluded, may have limited not only hydrolysis, but probably also prevented any degradation of the collagen in hybrid layers by salivary MMPs and/or bacterial contamination.

It is thought that, in perfect resin-dentin bonds, adhesive resins would be in intimate contact with the collagen fibrils in the hybrid layer. However, there is evidence that there may be a layer of water between the resin and the collagen fibrils, which have bound MMPs on their surfaces.[13] Chlorhexidine may act as a detergent to permit closer adaptation of resin to collagen fibrils and their associated MMPs,[13] as well as being a potent inhibitor of the most common MMPs in the dentin matrix.[14]

CONCLUSIONS

Under the conditions of the current study, in caries-free permanent teeth, the degradation of dentin hybrid layers created beneath occlusal-surface resin composites *in vivo*, using an acetone-based adhesive, is extensive at 12 months. This degradation appears to be greatly reduced, if not eliminated, by use of a chlorhexidine solution after etching. *In vitro* bond strength was not affected by this modification of the adhesive technique.

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