©Operative Dentistry, 2007, **32-2**, 107-111

Clinical Research

The Effect of Chlorhexidine on Dentin Hybrid Layers *In Vivo*

WW Brackett • FR Tay • MG Brackett
A Dib • RJ Sword • DH Pashley

Clinical Relevance

Degradation of dentin hybrid layers in Class I resin composite restorations is minimal over six months but is lessened by the application of 2% chlorhexidine digluconate after etching.

SUMMARY

This *in vivo* study evaluated by TEM the degradation of dentin hybrid layers in deep occlusal resin composite restorations. Caries-free premolars scheduled for extraction as part of orthodontic treatment were prepared, restored and evaluated after two and six months. The adhesive used was a single-bottle etch-and-rinse product (Single Bond Plus, 3M ESPE). Control group restorations were placed according to the manufacturer's instructions, while the experimental group received application of a 2% solution of chlorhexidine digluconate after etching. No degradation was observed in either group after two months. Slight degradation was found in the control group after six months, but none was observed in the experimental group. *In vitro* testing showed no significant difference in microtensile bond strength between the control and experimental adhesive protocols.

*William W Brackett, DDS, MSD, associate professor, Department of Oral Rehabilitation, School of Dentistry, Medical College of Georgia, Augusta, GA, USA

Franklin R Tay, BDSc, PhD, Department of Oral Biology and Maxillofacial Pathology, School of Dentistry, Medical College of Georgia, Augusta, GA, USA

Martha Goël Brackett, DDS, MSD, assistant professor, Department of Oral Rehabilitation, School of Dentistry, Medical College of Georgia, Augusta, GA, USA

Alejandro Dib, CD, MO, professor and director, Postgraduate General Dentistry, Facultad de Estomatologia, Benémerita Universidad Autónoma de Puebla, Puebla, Mexico

Rhoda J Sword, DMD, instructor, Department of Oral Rehabilitation, School of Dentistry, Medical College of Georgia, Augusta, GA, USA

David H Pashley, DMD, PhD, regent's professor, Department of Oral Biology and Maxillofacial Pathology, School of Dentistry, Medical College of Georgia, Augusta, GA, USA

*Reprint request: 1120 15th Street, Augusta, GA 30912, USA: e-mail: wbrackett@mail.mcg.edu

DOI: 10.2341/06-55

INTRODUCTION

The adhesion of resin to dentin became feasible with the advent of hydrophilic resins capable of infiltrating and polymerizing within the collagen mesh exposed through acid decalcification of dentin, forming a hybrid layer.[1] Unfortunately, this adhesion degrades over time,[2-3] probably due to hydrolysis of both the resin and

collagen.[4] The latter is promoted by metalloproteinase (MMP) enzymes in both saliva and dentin,[5] which may promote matrix degradation to slow the progression of caries through removal of the dentinal collagen exposed by demineralization.[6-7]

The rate at which this degradation occurs *in vivo*, and its consequences, have not been thoroughly studied. Using primate models, Koshiro and others[8] and Sano and others[3] have demonstrated subclinical microscopic degradation in hybrid layers over one year, although bond strength decreased only for an etch-and-rinse adhesive[8] and remained the same for the two self-etching primers that were evaluated. Hashimoto and others[2] have shown microscopic degradation and significant decreases in bond strength over one-to-three years when Class II resin composites were placed with an etch-and-rinse adhesive in carious deciduous molars. A similar study by Hebling and others[9] of Class I resin composites placed in carious deciduous molars demonstrated microscopic degradation of the hybrid layers after only six months. A study of deep Class I resin composites placed in non-carious third molars with self-etching primers demonstrated decreasing bond strength for one of the two adhesive systems used, with slight degradation of the hybrid layers of both over one year.[10]

Chlorhexidine is known to be an inhibitor of MMP activity *in vitro*.[11-12] However, in an *in vivo* study,[9] an experimental group included the application of a 2% solution of chlorhexidine diglucontate to primary dentin after acid etching. Teeth from this group showed less degradation of the hybrid layers than the control group, although the study evaluated only a small number of teeth.

The purpose of this study was to apply the protocol from Hebling and others[9] to a larger number of deep Class I resin composite restorations in caries-free permanent premolars to determine if the rate of degradation and the effect of chlorhexidine application is the same over six months in permanent teeth. The effect of the chlorhexidine application on immediate *in vitro* dentin bond strength of the etch-and-rinse 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, Puebla, Mexico. The study was conducted in accordance with all local regulations for the ethical treatment of human subjects.

Twelve pairs of like contralateral caries-free premolars, which had been scheduled for extraction as part of orthodontic treatment, were identified in six healthy patients with a median age of 15 years. Each patient received an occlusal cavity preparation approximately 3 mm in pulpal depth and 2.5 mm wide. The preparations were performed using a new 245 carbide bur (Brasseler USA, Savannah, GA, USA) at high speed under local anesthesia, rubber dam isolation and liberal air-water coolant. One premolar from each pair was randomly assigned to either the control or experimental group.

Restorations in the control group were preceded by 15-second etching of the preparations with 37% phosphoric acid gel (Scotchbond Etchant, 3M ESPE Dental Products, St Paul, MN, USA). After rinsing and brief air drying, a single-bottle adhesive (Adper Single Bond Plus, 3M ESPE) was applied three times, with agitation of the adhesive over 15 seconds, followed by thorough air drying and light curing for 10 seconds. To facilitate preparation of the microscopic specimens, a 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 (Filtek Supreme, 3M ESPE) that was placed in 2 mm thick increments, each of which was light cured for 40 seconds. Light output of the SL3000 curing light (3M ESPE) used 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 USA) and rubber polishing points (Enhance, Dentsply/Caulk, Milford, DE, USA).

The restoration of 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 using a new, dry pellet, leaving the dentin surface visibly moist. All the teeth were prepared and restored by the same operator.

After two and six months, respectively, half of the restored teeth in the subjects were extracted under local anesthesia. After extraction, the apical two-thirds of the roots were removed using a high-speed bur with water coolant to facilitate penetration of the 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; they were then processed for transmission electron microscopy.

Laboratory Procedures

All laboratory procedures were performed at the Medical College of Georgia, School of Dentistry, following that institution's regulations for the ethical treatment of human subjects.

Upon receipt of the specimens, 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 tetra-acetic acid. Demineralized epoxy resin-embedded 90-nm thick sections were prepared according to the TEM protocol of Tay and others,[13] which entails staining with 2% uranyl acetate and Reynolds' lead citrate for examination of the characteristics of the resin-dentin interfaces and 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 experimental teeth were examined.

In order to ensure that the use of chlohexidine did not impair the adhesion of resin to dentin, immediate microtensile bond strengths to dentin were determined on extracted teeth not derived from patients in the clinical part of the study. The same protocol and materials listed above were used, except that no layer of microfilled resin was placed, because the authors believed that the use of two resin composites of differing elastic modulus could compromise the bond strength data. A previously described non-trimming method was employed.[14] The sample size was five teeth/35 beams, with the teeth being sectioned one day after restoration and the beams being tested one day after sectioning. The type of failure was visually categorized at 2.5x magnification as either adhesive, cohesive or mixed. Bond strength was calculated, and data was obtained for the two statistically analyzed groups using a two-sample *t*-test at a confidence level of 5%.

RESULTS

Microtensile dentin bond strengths from both the experimental and control groups of specimens were found to be normally distributed, and no significant difference was found between them (p=0.11). Means (SD) were 58.3 (11.7) and 64.3 (15.2) MPa, respectively, and all failures were classified as adhesive.

None of the patients complained of post-operative sensitivity, and all restorations were clinically serviceable until the date of extraction. For teeth extracted after two months *in vivo*, no evidence of deterioration of the hybrid layers was evident in either the control or experimental groups (Figure 1). For teeth extracted after six months, no evidence of degradation was observed in the hybrid layers of restorations in the experimental group (Figure 2), but slight deterioration of the hybrid layers, manifesting as localized areas of debanding of collagen and detachment of the hybrid layers from underlying dentin, was evident in two of the six teeth comprising the control group (Figure 3).

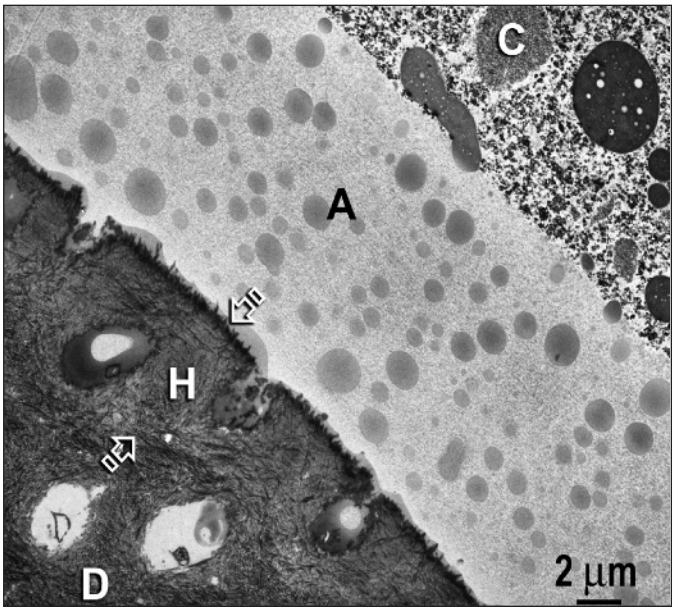

Figure 1. *Representative TEM of resin/dentin interface of both control and experimental teeth after two months* in vivo*. Integrity of the collagen is demonstrated by dark staining of collagen bands within dentin (D) and the hybrid layer (H; width indicated by broken arrows), which closely approximates the adhesive layer (A). (C = microfilled resin composite; original magnification = 5000x).*

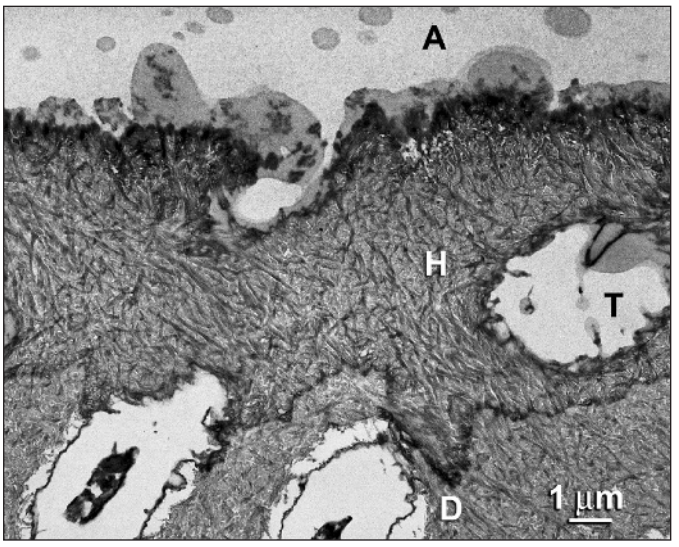

Figure 2. *Representative TEM of resin/dentin interface of experimental teeth after six months* in vivo*, demonstrating little change relative to two-month interval. Collagen within hybrid layer (H) retains banding. (A = adhesive layer; D = dentin; T = dentinal tubule; original magnification = 7500x).*

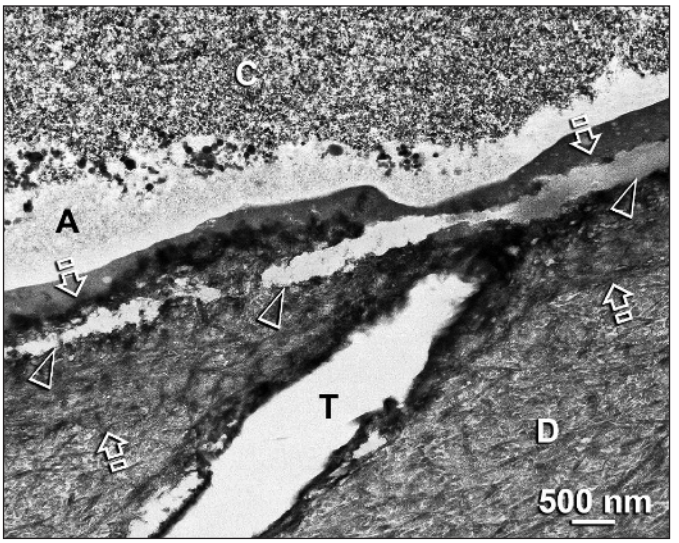

Figure 3. *Representative TEM of resin/dentin interface of two of the control teeth which demonstrated degradation of hybrid layers in some areas after six months* in vivo. *At increased magnification, the loss of collagen is evident as electron-lucent zone (triangular arrows) within the hybrid layer (broken arrows). (C = microfilled resin composite; A = adhesive layer; D = dentin; T = dentinal tubule; original magnification = 10,000x).*

DISCUSSION

The use of chlorhexidine solution after etching, as recommended by the manufacturer, does not appear to have detrimental effects on either the *in vitro* bond strength of the adhesive employed or on the clinical serviceability of Class I resin composite restorations over six months.

It appears that degradation of the hybrid layers in the caries-free permanent teeth that were evaluated progresses more slowly than degradation observed in carious deciduous teeth[2,9] and at about the same rate as in a previous study on non-carious permanent teeth.[10] In the opinion of the investigators, the degradation observed in this control group occurred more through host-derived MMPs acting on collagen than through the hydrolysis of resin, because the restored teeth 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.

The slower rate of degradation in this study, compared to a previous study that used the same protocol on deciduous teeth,[9] is probably not related to any difference in structure or composition between permanent and deciduous dentin. Although preparations in this study were deliberately extended into dentin near the pulp in which MMPs are more prevalent,[7] the relatively low pH of the etchant, which is known to inhibit MMP activity,[9] may have been more effective in this study than in carious deciduous teeth where dentinal MMPs had presumably already been activated by previous carious activity. Similarly, the ideal circumstances under which restorations could be placed in this study, whereby saliva was completely excluded, may have limited not only hydrolysis, but may also have prevented any degradation of collagen in the hybrid layers by salivary MMPs.

CONCLUSIONS

In caries-free permanent teeth, degradation of the dentin hybrid layers beneath Class I resin composites *in vivo* is not extensive at six months; however, it appears to be slowed by the use of a chlorhexidine solution after etching.

Acknowledgements

The authors gratefully acknowledge the financial support of the Facultad de Estomatología, Benémerita Universidad Autónoma de Puebla.

(Received 10 April 2006)

References

1. Nakabayashi N, Kojima K & Masuhara E (1982) The promotion of adhesion by the infiltration of monomers into tooth substrates *Journal of Biomedical Materials Research* **16(3)** 265-273.
2. Hashimoto M, Ohno H, Kaga M, Endo K, Sano H & Oguchi H (2000) *In vivo* degradation of resin-dentin bonds in humans over 1 to 3 years *Journal of Dental Research* **79(6)** 1385-1391.
3. Sano H, Yoshikawa T, Pereira PN, Kanemura N, Morigami M, Tagami J & Pashley DH (1999) Long-term durability of dentin bonds made with a self-etching primer, *in vivo* *Journal of Dental Research* **78(4)** 906-911.
4. Hashimoto M, Ohno H, Kaga M, Endo K, Sano H & Oguchi H (2001) Resin-tooth adhesive interfaces after long-term function *American Journal of Dentistry* **14(4)** 211-215.
5. Sano H (2006) Microtensile testing, nanoleakage, and biodegradation of resin-dentin bonds *Journal of Dental Research* **85(1)** 11-14.
6. Tjaderhane L, Larjava H, Sorsa T, Uitto VJ, Larmas M & Salo T (1998) The activation and function of host matrix metalloproteinases in dentin matrix breakdown in caries lesions *Journal of Dental Research* **77(8)** 1622-1629.
7. Chaussain-Miller C, Fioretti F, Goldberg M & Menashi S (2006) The role of matrix metalloproteinases (MMPs) in human caries *Journal of Dental Research* **85(1)** 22-32.
8. Koshiro K, Inoue S, Sano H, De Munck J & Van Meerbeek B (2005) *In vivo* degradation of resin-dentin bonds produced by a self-etch and an etch-and-rinse adhesive *European Journal of Oral Science* **113(4)** 341-348.
9. Hebling J, Pashley DH, Tjaderhane L & Tay FR (2005) Chlorhexidine arrests subclinical degradation of dentin hybrid layers *in vivo* *Journal of Dental Research* **84(8)** 741-746.

10. Donmez N, Belli S, Pashley DH & Tay FR (2005) Ultrastructural correlates of *in vivo*/*in vitro* bond degradation in self-etch adhesives *Journal of Dental Research* **84(4)** 355-359.
11. Pashley DH, Tay FR, Yiu C, Hashimoto M, Breschi L, Carvalho RM & Ito S (2004) Collagen degradation by host-derived enzymes during aging *Journal of Dental Research* **83(3)** 216-221.
12. Gendron R, Grenier D, Sorsa T & Mayrand D (1999) Inhibition of the activities of matrix metalloproteinases 2, 8, and 9 by chlorhexidine *Clinical and Diagnostic Laboratory Immunology* **6(3)** 437-439.
13. Tay FR, Pashley DH & Yoshiyama M (2002) Two modes of nanoleakage expression in single-step adhesives *Journal of Dental Research* **81(7)** 472-476.
14. Brackett WW, Ito S, Tay FR, Haisch LD & Pashley DH (2005) Microtensile dentin bond strength of self-etching resins: Effect of a hydrophobic layer *Operative Dentistry* **30(6)** 733-738.