Effect of Chlorhexidine Application in a Self-etching Adhesive on the Immediate Resin-Dentin Bond Strength

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Purpose: To investigate whether the application of chlorhexidine in a two-step self-etching adhesive has an adverse effect on the immediate resin-dentin bond strength.

Materials and Methods: Different amounts of 20 wt% chlorhexidine digluconate were added directly to the Clearfil SE Bond primer to prepare mixtures of 4 different concentrations of chlorhexidine: 0.05 wt%, 0.1 wt%, 0.5 wt%, and 1.0 wt%. Sixteen extracted third molars were randomly divided into 4 groups. Each group corresponded to one of the 4 chlorhexidine concentrations. Each of the 16 teeth was sectioned into halves. One half was customarily bonded with Clearfil SE Bond without chlorhexidine, and the other half was bonded with Clearfil SE Bond containing different concentrations of chlorhexidine. Microtensile bond strengths were tested immediately after specimen preparation. The modes of fractures were examined under a stereomicroscope.

Results: No significant difference of immediate resin-dentin bond strength was observed between the control groups and any of the experimental groups containing chlorhexidine ($p > 0.05$).

Conclusion: The addition of chlorhexidine to a two-step self-etching adhesive primer (Clearfil SE Bond primer) has no adverse effect on the immediate resin-dentin bond strength when the chlorhexidine concentration in the primer is lower than or equal to 1.0 wt%.

Keywords: chlorhexidine, bond degradation, matrix metalloproteinases, self-etching adhesives, microtensile bond test.

J Adhes Dent 2010; 12: 27-31.
doi: 10.3290/j.jad.a17543

Submitted for publication: 28.05.08; accepted for publication: 16.10.08.

Bonds created by dentin adhesives are not as durable as previously conjectured.[2,7,12,15] The susceptibility of contemporary dentin adhesives to water/oral fluid sorption, polymer swelling, and consequent resin leaching has been proven by many in vitro and in vivo studies.[12,18] Apart from these extrinsic factors, intrinsic, host-derived matrix metalloproteinases (MMPs) also appear to be involved in the breakdown of hybrid layers.[19,26]

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Dentin matrix contains MMPs, a class of zinc-activated, calcium-dependent endopeptidases, which play strategic roles in tooth development[5] and dentinal caries.[10] MMPs are a group of 23 mammalian enzymes capable of degrading all extracellular matrix components. Human dentin contains at least collagenase (MMP-8), gelatinases (MMP-2 and MMP-9), and enamelysin (MMP-20).[21,22,31,32] It has been shown that simplified etch-and-rinse adhesives[23] and the less aggressive versions of self-etching adhesives[25,33] are capable of releasing and activating endogenous MMPs during dentin bonding, which are thought to be responsible for the manifestation of thinning and disappearance of collagen fibrils from incompletely infiltrated hybrid layers in aged, bonded dentin.[3,16,26]

The activity of MMPs can be suppressed by protease inhibitors,[26] indicating that MMP inhibition may be beneficial in the preservation of hybrid layers. This has been demonstrated in recent in vivo and in vitro studies in which the application of chlorhexidine, known to have a broad-spectrum MMP-inhibitory effect,[14] significantly improved the integrity of the hybrid layers created by a simplified etch-and-rinse adhesive (Single Bond; 3M ESPE; St Paul, MN, USA).[6,8,9,19] However, whether chlorhexidine can be used in self-etching adhesives to preserve dentin bonds has not been reported. To confirm that chlorhexidine can be used in self-etching adhesives, the first step is to ensure that the use of chlorhexidine in self-etching adhesives does not impair the immediate bond strength of resin to dentin. The purpose of this study was to evaluate the effect of chlorhexidine application in a two-step self-etching adhesive on the immediate in vitro bond strength. The null hypothesis tested was that the use of chlorhexidine in a two-step self-etching adhesive (Clearfil SE Bond) does not impair the immediate bond strength of resin to dentin.

MATERIALS AND METHODS

Preparation of a Self-etching Adhesive Primer Containing Chlorhexidine

A two-step self-etching adhesive (Clearfil SE Bond, Kuraray; Osaka, Japan) was selected for this study. Different amounts of 20 wt% chlorhexidine digluconate were added directly to the Clearfil SE Bond primer to prepare mixtures containing 4 different concentrations of chlorhexidine: 0.05 wt%, 0.1 wt%, 0.5 wt%, and 1.0 wt%.

Tooth Preparation

Sixteen unerupted, caries-free third molars were collected from patients from whom informed consent was obtained. The study protocol was reviewed and approved by the Ethics Committee for Human Studies, Peking University, Beijing, China. These molars were stored in 0.9% NaCl containing 0.02% sodium azide at 4°C for no more than one month. The 16 teeth were randomly divided into 4 groups. Each group corresponded to 1 of the following 4 concentrations of chlorhexidine: 0.05 wt%, 0.1 wt%, 0.5 wt%, and 1.0 wt%.

The occlusal enamel of each tooth was removed. Each tooth was hemisected faciolingually into halves using a slow-speed water-cooled saw equipped with a diamond-impregnated disk (Isomet 1000; Buehler; Lake Bluff, IL, USA). Each half was randomly assigned to the control group or an experimental group. The teeth were polished on a wet 600-grit silicon carbide paper by hand for 30 s to create a realistic smear layer on the surface of the occlusal mid-coronal dentin.

Bonding Procedures

The control group teeth were bonded with Clearfil SE Bond without chlorhexidine, according to the manufacturer's instructions. The teeth in experimental groups were bonded with Clearfil SE Bond containing different concentrations of chlorhexidine. Five or 6 increments of resin composite (Clearfil AP-X, Kuraray; Osaka, Japan) were obliquely added to the bonded surfaces and individually light cured for 20 s using a halogen light-curing unit with an output of 700 mW/cm^2. The teeth were then stored in distilled water at 37°C for a week.

Microtensile Bond Testing

The teeth were longitudinally sectioned across the bonded interface in sections perpendicular to the bonded surfaces with a diamond saw (Isomet 1000, Buehler), to produce a series of 0.9 mm x 2 mm x 8 mm beams. Three to 4 beams were obtained from each preparation. These beams were then trimmed into hourglass-shaped specimens with a cross-sectional area of approximately 0.6 mm^2. These specimens were stored in distilled water and tested after 24 h. Each specimen was individually fixed to a custom-made testing jig with a cyanoacrylate glue (Universal instant adhesive; Henkel Adhesives; Shantou, China). The specimen was then subjected to tensile loading at a crosshead speed of 1.0 mm/min until failure (Autograph DCS-5000, Shimadzu; Kyoto, Japan).

Debond Pathway Determination

Both surfaces of each fractured specimen were observed under a stereomicroscope (Olympus 220670; Tokyo, Japan) at 40X magnification to record the failure modes. The fracture modes were classified as follows: (1) cohesive failures in the composite resin or adhesive resin; (2) failures in the adhesive joint; (3) cohesive failures in dentin; and (4) mixed failures.

Statistical Analysis

Two-way analysis of variance (ANOVA) and post-hoc Tukey tests were used to compare the effects of the dentin treatments (control vs chlorhexidine) on bond strengths. Statistical significance was pre-set at $\alpha = 0.05$.The statistical unit was beams, not teeth.

RESULTS

Microtensile Bond Strengths

From a total of 126 specimens, only 2 specimens failed during the pre-test phase, and they were statistically treated as missing data. The means and standard deviations of the microtensile bond strengths (µTBS) are summarized in Table 1. None of the experimental groups with different chlorhexidine concentrations showed a significant difference to the control group in terms of the immediate bond strength ($p > 0.05$). The mean µTBS of the control group was 68.08 MPa and that of the experimental group was 68.60 MPa.

Distribution of the Failure Mode

Table 2 summarizes the distribution of the failure modes. Cohesive failures in the composite resin or adhesive resin were the most common fracture pattern observed in both the control and experimental groups, followed by cohesive failures in the dentin and mixed failures. There were few adhesive joint failures. All mixed failures were the combination of cohesive failures in resin and failures in the adhesive joint.

DISCUSSION

No difference was observed between the control and experimental groups with regard to the immediate bond

Table 1 Immediate in vitro microtensile bond strengths (MPa)

Control/experimental group	Concentration of chlorhexidine	Sample size (n)	µTBS (SD)
Control	0	15	66.49 (8.98)
Chlorhexidine	0.05%	16	64.75 (9.46)
Control	0	15	70.62 (11.83)
Chlorhexidine	0.10%	15	69.81(15.41)
Control	0	15	69.75(3.71)
Chlorhexidine	0.50%	16	70.97(3.16)
Control	0	16	65.60 (12.71)
Chlorhexidine	1.00%	16	68.96 (14.38)
Total	control	61	68.08 (12.02)
	chlorhexidine	63	68.60 (13.02)

Table 2 Distribution of failure modes

control /experimental group		Failure mode			
		Cohesive failures in the composite resin or adhesive resin	Failures in the adhesive joint	Cohesive failures in dentin	Mixed failures
Control		12/15	0/15	1/15	2/15
chlorhexidine (0.05%)		13/16	0/16	1/16	2/16
Control		14/15	0/15	1/15	0/15
chlorhexidine (0.1%)		10/15	0/15	4/15	1/15
Control		12/15	0/15	1/15	2/15
chlorhexidine (0.5%)		14/17	1/17	2/17	0/17
Control		10/16	1/16	2/16	3/16
chlorhexidine (1.0%)		10/16	1/16	3/16	2/16
Total	Control	48/61	1/61	5/61	7/61
	Chlorhexidine	47/64	2/64	10/64	5/64

strength of resin to dentin. Therefore, we can conclude that the addition of chlorhexidine to Clearfil SE Bond primer has no adverse effect on the immediate bond strength of resin to dentin when the concentration of chlorhexidine in the adhesive primer is lower than or equal to 1.0 wt%.

Chlorhexidine has been widely used as an antimicrobial agent, eg, as a cavity disinfectant before the placement of restorations. Previous studies have demonstrated that as a cavity disinfectant, chlorhexidine application before or after acid etching has no adverse effects on immediate bond strength of etch-and-rinse adhesives.[11,13,27,28,30] Recent studies have also examined the use of chlorhexidine after acid etching as an MMP inhibitor to preserve dentin hybrid layers, demonstrating initial bond strengths comparable with those of the controls.[8] However, the direct addition of chlorhexidine in self-etching adhesive primers as an MMP inhibitor has not been reported previously. Analysis of the present data indicates that chlorhexidine application in a two-step self-etching adhesive (Clearfil SE Bond) has no negative effect on the bond strength of resin to dentin. Nevertheless, in this study, we only applied chlorhexidine in Clearfil SE Bond. We have to point out that chlorhexidine may not be beneficial to all two-step self-etching adhesives because the chemistry varies for each adhesive.

It has been reported that even at very low concentrations, chlorhexidine possesses desirable MMP-inhibitory properties. Chlorhexidine at concentrations as low as 0.03% can completely inhibit MMP-2 and MMP-9 gelatinase activity.[14] Therefore, we selected a chlorhexidine concentration of 0.05% as one of the test chlorhexidine concentrations. To determine the optimum concentration of chlorhexidine in the Clearfil SE Bond primer that can effectively preserve the dentin hybrid layers and have the least adverse effect on the immediate bond strength, chlorhexidine in concentrations of 0.05%, 0.1%, 0.5%, and 1.0% were tested. However, chlorhexidine has no negative effect on immediate bond strength, even at a concentration as high as 1.0%. Since very low concentrations of chlorhexidine can inhibit MMPs effectively, testing higher concentrations of chlorhexidine is not required.

In recent studies in which chlorhexidine was used as an MMP inhibitor in etch-and-rinse adhesives, 2% chlorhexidine solution was applied after acid etching. Excess chlorhexidine was blot dried or removed with absorbent papers prior to the application of adhesive and resin composite.[6,8,9,19] It is true that chlorhexidine can inactivate MMPs when applied after acid etching; however, the application of etch-and-rinse adhesives might subsequently further activate the MMPs to some degree. This may occur because etch-and-rinse adhesives have low acidity, which can further demineralize dentin and activate MMPs by "acid-activation" mechanism.[34] Nevertheless, in our present study, chlorhexidine was added directly to the self-etching adhesive primer. When the acidic monomers in the self-etching adhesives demineralize dentin and activate MMPs, chlorhexidine can simultaneously inactivate MMPs until the adhesive is light cured. Therefore, we hypothesize that the use of chlorhexidine in self-etching adhesives applied in this manner might result in the better preservation of dentin hybrid layers than the use of 2% chlorhexidine solution in etch-and-rinse adhesives, which is applied after acid-etching. However, it is possible that chlorhexidine could also be incorporated directly in the two-step etch-and-rinse adhesives, which might bring the same benefits. Additionally, chlorhexidine might also be incorporated in the primer of three-step etch-and-rinse adhesives to preserve dentin bonding. All these hypotheses have to be tested in further studies.

The ability of μTBS methods to produce fractures in the adhesive joint has been described as a distinct advantage over traditional strength-based testing methods by previous investigators.[24,29,35] However, studies to date have shown that the μTBS testing method was unable to consistently produce joint failures in the short-term storage group, especially when the bond strength was high.[4,20] As the adhesive joint deteriorates over time, the bond strength decreases, and the rate of failures in the adhesive joint becomes higher. Therefore, long-term μTBS data may more closely represent the strength of the adhesive joint, whereas short-term data may represent the strength of the substrate.[1,4,17] Failure analysis of our present study, which showed predominant failure in the composite resin or adhesive resin, was in agreement with these previous studies. This was possibly because of the high immediate bond strengths obtained in our present study, which on average were higher than 68 MPa.

Although the application of chlorhexidine in Clearfil SE Bond has no adverse effect on the immediate bond strength, as is shown in our present study, there are three main outcomes possible when chlorhexidine is added into Clearfil SE Bond primer. First, chlorhexidine does not react with any components of the Clearfil SE Bond primer, keeping its inhibitory activity against MMPs, and would help preserve the dentin bond. Second, chlorhexidine does react with some components of Clearfil SE Bond primer, but its inhibitory activity against MMPs is not impaired, and it would preserve the dentin bond as well. Third, chlorhexidine reacts with some components of Clearfil SE Bond primer and at the same time, its inhibitory activity against MMPs is impaired. Although it has no negative effect on bond strength because of its very low concentrations, it would not preserve dentin bond as expected.

For the successful application of chlorhexidine in self-etching adhesives to preserve dentin bond, further studies have to be conducted to confirm that: 1. when added into self-etching adhesive primer, chlorhexidine still maintains its inhibitory activity against MMPs; 2. the hybrid layers obtained by using self-etching adhesives containing chlorhexidine exhibit normal structural integrity of the collagen network after a period of aging; 3. when chlorhexidine is used in self-etching adhesives, a significantly lower reduction in bond strength is observed over a period of aging.

CONCLUSIONS

Within the limitations of this study, the conclusion can be drawn that added directly to a two-step self-etching adhesive primer (Clearfil SE Bond primer), chlorhexidine has no adverse effect on the immediate resin-dentin bond strength when the chlorhexidine concentration in the adhesive primer is lower than or equal to 1.0 wt%. The null hypothesis advanced in this study was accepted.

ACKNOWLEDGMENTS

The authors are grateful to Kuraray for the generous donation of Clearfil SE Bond. We are also grateful to Mr Zhihui Sun and Mr Wei Bai (Department of Dental Materials, Peking University School and Hospital of Stomatology, Beijing, P. R. of China) for their assistance in the microtensile bond test.

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Clinical relevance: This study proved that the use of chlorhexidine in a two-step self-etching adhesive (Clearfil SE Bond) does not impair the immediate resin-dentin bond strength. Further studies have to be performed to confirm the successful use of chlorhexidine in self-etching adhesives to preserve the dentin bond.