

***In Vivo* and *In Vitro* Effects of Chlorhexidine Pretreatment on Immediate and Aged Dentin Bond Strengths**

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Clinical Relevance

For the tested etch-and-rinse and self-etch adhesives, 2%chlorhexidine application can counteract time-dependent decline in adhesive bonds to dentin, and increase the bonding effectiveness over time.

SUMMARY

This study evaluated the effect of 2% chlorhexidine (CHX) pretreatment of dentin on the immediate and aged microtensile bond strength (μ TBS) of different adhesives to dentin *in vivo* and *in vitro*. Class I cavities were prepared in 80 caries-free human third molars of 40 patients in a split-mouth fashion. In each tooth pair, one tooth received 2% CHX pretreatment after which both teeth were randomly assigned to one of the following groups

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with respect to the type of adhesive system applied: Adper Single Bond 2 (etch-and-rinse), Clearfil SE Bond (two-step self-etch), Clearfil S³ Bond (one-step self-etch), and Adper Prompt-L-Pop (all-in-one self-etch). The teeth were restored with resin composite and extracted for μ TBS testing either immediately or after six months in function. *In vitro* specimen pairs were prepared as with the clinical protocol in intact, freshly extracted human molars, and thereafter, subjected to testing immediately or after 5000 \times thermocycling. Data were analyzed with four-way analysis of variance (ANOVA). Bonferroni test was utilized for pair-wise comparisons. The immediate bond strength values were significantly higher than “aged” ones for all tested adhesives ($p=0.00$). The *in vitro* immediate bond strength values were statistically higher than *in vivo* bond strength values ($p<0.05$). While the bond strength of *in vitro* aged, CHX-treated samples were higher than their *in vivo* counterparts ($p<0.05$), no difference was observed in non-CHX treated groups ($p>0.05$). In the

absence of CHX pretreatment, all adhesives showed significantly higher immediate bond strength values than CHX-treated groups, while all “aged”, non-pretreated adhesives exhibited significantly lower bond strength values (both $p < 0.05$). By contrast, chlorhexidine pretreatment resulted in significantly higher aged bond strengths, regardless of the adhesive system and testing condition. Aging-associated decline in dentin bond strength of etch-and-rinse and self-etch adhesives can be counteracted by chlorhexidine application.

INTRODUCTION

Resin-dentin bonds are less durable than resin-enamel bonds, which might be one reason for the relatively short lifespan of tooth-colored fillings.¹ Degradation of the resin-dentin bond has been associated with water/oral fluid sorption, polymer swelling, and resin leaching *in vitro* and *in vivo*.^{2,3} Endogenous matrix metalloproteinases (MMPs) also appear to be involved in the disintegration of hybrid layers over time.^{2,4-6} Collagen fibrils exposed during the acid etching procedure become susceptible to hydrolytic and enzymatic degradation, a process mediated by activation of dentin MMPs.⁴ Likewise, etch-and-rinse adhesives and self-etching adhesives can activate endogenous MMPs during dentin bonding.⁷⁻⁹

Chlorhexidine (CHX) is a nonspecific protease inhibitor¹⁰ that can suppress collagenolytic/gelatinolytic activity of dentin matrices.⁴ As CHX has cationic properties, it can bind electrostatically to negative carboxyl groups and hydroxyl groups of collagen and noncollagenous phosphoproteins in demineralized dentin, as well as electrostatically to phosphate groups in hydroxyapatite crystallites in mineralized dentin. Application of CHX at different concentrations has been shown to maintain the hybrid layer preservation in several *in vivo*¹¹⁻¹³ and *in vitro* studies.¹⁴⁻¹⁶ Based on published data, there appears to be an increasing tendency among clinicians to apply CHX on dentin prior to bonding procedures. It has been recommended to apply CHX on etched dentin in association with etch-and-rinse adhesives, before primer and bonding application.¹⁷ The binding of CHX to demineralized dentin is much higher than that to mineralized dentin.¹⁸ There are many results of *in vitro* studies about CHX application before bonding procedure,^{14-16,19-22} but these results are yet to be verified *in vivo*. However, the effect of CHX on self-etching adhesives has not been well determined.

Based on these observations, the aim of this clinical and laboratory study was to test the efficacy of 2% CHX pretreatment on the microtensile bond strength (μ TBS) of different adhesives to dentin immediately and after aging. The null hypotheses tested were that: 1) CHX pretreatment has no influence on μ TBS values to dentin, 2) bond strength is not influenced by *in vivo* or *in vitro* testing, and 3) bond strength values are not related to the tested adhesive systems.

METHODS AND MATERIALS

This study was conducted in both laboratory and clinical phases. The *in vitro* and clinical research protocols and consent forms were evaluated and approved by the Institutional Human Subject Review Committee.

In Vitro Procedures

Eighty freshly extracted intact human third molars were stored in distilled water for up to 30 days. Standardized Class I cavity preparations with 3-mm mesial-distal width, 2-mm buccolingual width, and 2-mm depth were completed using a diamond cylinder bur (Diatech, Swiss Dental Instruments, Heerbrugg, Switzerland) in a water-cooled high-speed handpiece.

The teeth were randomly divided into four groups ($n=20$) with respect to the type of adhesive system tested: group I, two-step etch-and-rinse (Adper Single Bond 2, 3M ESPE, St Paul, MN, USA); group II, two-step self-etch (Clearfil SE Bond, Kuraray, Tokyo, Japan); group III, one-step self-etch (Clearfil S³ Bond, Kuraray); and group IV, all-in-one self-etch, (Adper Prompt-L-Pop, 3M ESPE). Prior to adhesive application, specimens in each group were further randomly allocated into two subgroups ($n=10$): (A) no CHX digluconate pretreatment and (B) 2% CHX digluconate pretreatment. In subgroups B, 2% CHX was applied for 30 seconds using a foam pellet saturated with the solution. Excess CHX was blot-dried prior to application of the adhesive. In group I, CHX was applied after the acid-etching procedure, while in groups II, III, and IV, the cavities were treated with CHX prior to the application of the tested self-etch adhesives. In all groups, the cavities were restored with a hybrid resin composite (Filtek Z250, 3M ESPE). The composite was placed in 2-mm thick increments, each light cured with a LED unit for 20 seconds. The main components, modes of application, and batch numbers of the materials used in the present study are shown in Table 1.

Table 1. Materials, Manufacturers, Batch Numbers, Chemical Composition, and Application Modes		
Materials (Batch Number)	Composition	Mode of Application
Adper Single Bond 2 (3M ESPE, MN, USA) (51202)	Etchant: Scotchbond acid-35% phosphoric acid	Apply Scotchbond etchant to enamel and dentin. Wait 15 s. Rinse for 10 s. Blot excess water leaving tooth moist.
	Bis-GMA, HEMA, co-polymer of acrylic/itaconic acids, diurethane dimethacrylate, glyceroldimethacrylate, water, and ethanol	Adhesive: Using a fully saturated brush tip for each coat, apply two consecutive coats of Adper Single Bond adhesive to etched enamel and dentin. Dry gently for 2-5 s. Light cure for 10 s.
Clearfil SE Bond (Kuraray Medical Inc, Okayama, Japan) (00972A - Primer) (01443A - Bond)	Primer: MDP, HEMA, dimethacrylate monomer, water, catalyst	Prime for 20 s (no mixing required)
	Bond: MDP, HEMA, dimethacrylate monomer, microfiller, catalyst	Apply Bond and light-cure for 10 s.
Clearfil S ³ (Kuraray Medical Inc, Okayama, Japan) (00143A)	MDP, Bis-GMA, HEMA, dl-camphoroquinone, ethanol, water, colloidal silica	Apply bond and wait 20 s. Dry with high-pressure air for 5 s. Light-cure for 10 s.
Adper Prompt-L-Pop (3M ESPE, MN, USA) (387690)	Methacrylated phosphoric esters, Bis-GMA, camphoroquinone, stabilizers, water, 2- (HEMA), polyalkenoic acid	Mix adhesive according to instructions. Apply adhesive with a rubbing motion for 15 s. Gently but thoroughly air-dry to remove the aqueous solvent. Light-cure for 10 s. Apply a second coat. Gently but thoroughly air-dry to remove the aqueous solvent. Light-cure for 10 s.
Klorhex (Drogsan Medicine, Ankara, Turkey) (2006/66)	2% Chlorhexidine digluconate	Use a foam pellet saturated with the solution for 30 s. Blot-dry excess CHX.
Filtek Z250 (3M ESPE, MN, USA) (N110475)	Bis-GMA, UDMA, Bis-EMA resin, zirconium, silica	Insert incrementally in 2-mm increments. Light-cure for 40 s.
Abbreviations: BisEMA, ethoxylated bisphenol-A-glycidyl methacrylate; Bis-GMA, bis-phenol A diglycidylmethacrylate; HEMA, 2-hydroxyethyl methacrylate; MDP, 10-methacryloyloxydecyl dihydrogen phosphate; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate.		

Restored teeth were stored in distilled water for 24 hours, after which finishing and polishing procedures were accomplished with diamond burs (Dia-tech) and flexible disks (Sof-Lex Pop-on, 3M ESPE), respectively. In each subgroup, half of the specimens (n=5) were randomly assigned for immediate bond strength testing (ie, after 24 hours), while the other half was subjected to thermocycling (5000X) in a water bath from 5°C to 55°C with a dwell time of 30 seconds at each temperature and a transfer time of 5 seconds. All specimens were stored in distilled water until bond strength testing.

In Vivo Procedures

Forty 23- to 28-year-old healthy volunteers with a pair of occluding, noncarious, contralateral third molars scheduled for future extraction were enrolled for *in vivo* sample preparation. Class I cavities conforming to the dimensions of the *in vitro* counterparts (3 mm mesial-distal, 2 mm buccolingual, 2 mm deep) were prepared in third molars under rubber dam isolation. As with the *in vitro* part, patients were randomly allocated into four groups with respect to the adhesive system applied:

group V, two-step etch-and-rinse (Adper Single Bond 2); group VI, two-step self-etch (Clearfil SE Bond); group VII, one-step self-etch (Clearfil S³ Bond); and group VIII, all-in-one self-etch, (Adper Prompt-L-Pop). For each tooth pair, one tooth received adhesive placement without prior CHX application, while the contralateral molar was pretreated with 2% CHX in accordance with the *in vitro* specimen preparation protocol. Following adhesive application, the cavities were restored with a hybrid resin composite (Filtek Z250) as with the *in vitro* specimens, and finishing and polishing procedures were performed after 24 hours. Half of the patients' teeth were extracted after 24 hours for evaluation of immediate bond strength, while the remaining teeth were periodically monitored and extracted six months later. Extracted teeth were stored in distilled water at room temperature until bond strength evaluation.

Microtensile Bond Test

Restored teeth were longitudinally sectioned across the bonded interface using a water-cooled diamond saw in a precision cutting machine (Micracut 201,

Table 2. Microtensile Bond Strength Values (in MPa) and SD (\pm) of Tested Adhesive Systems (n=5)

Adhesive Systems	Time	Chlorhexidine	In Vitro Mean \pm SD	In Vivo Mean \pm SD
Adper Single Bond 2 (SB)	Immediate	CHX (–)	36.13 \pm 2.46	28.45 \pm 2.25
		CHX (+)	31.36 \pm 1.29	24.37 \pm 1.32
	Aged	CHX (–)	17.60 \pm 1.71	16.33 \pm 0.67
		CHX (+)	23.91 \pm 1.23	19.87 \pm 0.66
Clearfil SE (SE)	Immediate	CHX (–)	35.38 \pm 2.26	27.13 \pm 2.04
		CHX (+)	32.94 \pm 1.72	24.14 \pm 1.07
	Aged	CHX (–)	17.97 \pm 1.31	16.20 \pm 0.52
		CHX (+)	23.68 \pm 1.67	20.11 \pm 1.36
Clearfil S ³ (S ³)	Immediate	CHX (–)	30.23 \pm 1.20	24.95 \pm 1.01
		CHX (+)	27.5 \pm 1.54	22.98 \pm 0.82
	Aged	CHX (–)	14.36 \pm 1.16	12.81 \pm 1.00
		CHX (+)	19.83 \pm 0.78	16.08 \pm 0.68
Adper Prompt-L-Pop (PLP)	Immediate	CHX (–)	27.11 \pm 0.96	24.18 \pm 0.22
		CHX (+)	23.69 \pm 1.12	20.40 \pm 0.71
	Aged	CHX (–)	12.96 \pm 0.49	10.84 \pm 1.16
		CHX (+)	17.20 \pm 0.65	14.02 \pm 0.56

MetLab Inc, Niagara Falls, NY, USA) to obtain bonded sticks with a cross-sectional area of approximately 1 mm². The exact dimension of each beam was measured using a digital caliper. Slabs sectioned from the center of each composite restoration were selected. Three beams were selected from each tooth, resulting in 15 beams for each subgroup. Each beam was attached to the test apparatus with a cyanoacrylate adhesive and stressed to failure under tension using a universal testing machine (Micro Tensile Tester, Bisco, Schaumburg, IL, USA) with a 50 kgf load cell and a crosshead speed of 1 mm/min.

The fractured specimens were examined using a stereomicroscope (SZ 61, Olympus, Tokyo, Japan) at 25 \times magnification. The failure modes were classified and recorded as cohesive (failure within the composite resin or within dentin), adhesive (failure across the bonding interface), or mixed failure.

Scanning Electron Microscopic Evaluation

Two specimens were randomly selected from each group to evaluate the effect of CHX application on resin-dentin interface. As a preparation for scanning electron microscopic evaluation, specimens were first etched with 37% phosphoric acid for 15 seconds followed by 30 seconds rinsing with water. The specimens were fixed in 10% neutral buffered formalin for 24 hours and rinsed in running water for 15 minutes. Then, the specimens were dehydrated in ascending grades of ethanol (25% for 20 minutes, 50% for 20 minutes, 75% for 20 minutes, 95% for 20 minutes, and 100% for 60 minutes) and

thereafter, were immersed in hexamethyldisilazane for 10 minutes. Finally, the specimens were placed on a filter paper inside a covered glass vial, and air-dried at room temperature for 12 hours. The specimens were sputter-coated with gold and examined through scanning electron microscopy (SEM).

Statistical Analysis

Statistical analysis of the data was made using four-way analysis of variance (ANOVA). Bonferroni test was utilized for pair-wise comparisons. Results of failure modes were subjected to nonparametric analysis using Pearson chi-square test. All results were analyzed with SPSS 18.0 for Windows (SPSS Inc, Chicago, IL, USA), with the level of significance set at $p < 0.05$.

RESULTS

The μ TBS values of test groups are presented in Table 2 as means and standard deviation. All tested variables (type of adhesive, CHX pretreatment, aging, and *in vivo/in vitro* testing), significantly influenced the dentin bond strength ($p < 0.05$, four-way ANOVA).

For all tested adhesives, the immediate bond strength values were significantly higher than “aged” ones ($p = 0.00$). While CHX pretreated groups showed significantly lower immediate bond strength values ($p < 0.05$), they yielded higher bond strength values in aged samples ($p < 0.05$) (Table 3). There were statistically significant differences between *in vitro* and *in vivo* immediate dentin bond strength

Table 3. Results for the Comparison of Means (MPa) of the CHX (+) vs CHX (–) Within the Levels of the Test Conditions, Adhesive Systems, and Time ($\alpha=0.05$).			
Test Condition	Adhesive Systems	Time	p-Value
In vitro	SB	Immediate	0.000
		Aged	0.000
	SE	Immediate	0.003
		Aged	0.000
	S ³	Immediate	0.001
		Aged	0.000
	PLP	Immediate	0.000
		Aged	0.000
In vivo	SB	Immediate	0.000
		Aged	0.000
	SE	Immediate	0.000
		Aged	0.000
	S ³	Immediate	0.003
		Aged	0.000
	PLP	Immediate	0.000
		Aged	0.000

values for all tested adhesives regardless of CHX pretreatment ($p<0.05$). While there were significant differences between *in vitro* and *in vivo* aged CHX treated samples ($p<0.05$), no difference was observed in non-CHX treated samples ($p>0.05$) (Table 4). The fracture mode was predominantly adhesive and mixed, and was similar among the experimental groups ($p>0.05$).

In Vitro Specimens

In the absence of CHX pretreatment, immediate and aged dentin bond strength values were similar for SB and SE, and for S³ and PLP ($p>0.05$), with the immediate and aged dentin bond strength of SB and SE being significantly higher than S³ and PLP ($p<0.05$). CHX pretreatment caused significant differences in immediate dentin bond strength in all groups except between SB and SE ($p=0.336$). Significant differences were also observed in “aged” CHX-pretreated groups, except between SB and SE, and S³ and PLP ($p>0.05$) (Table 5).

In Vivo Specimens

In experimental groups that did not receive prior CHX treatment, results were similar as with their *in vitro* counterparts, with the exception of insignificance between SE and S³. For CHX-pretreated samples, immediate bond strength values were similar among groups with the exception of PLP,

Table 4. Results For The Comparison of Means (MPa) of The In Vitro vs In Vivo Test Conditions Within The Levels of The Adhesive Systems, Time, And CHX Application			
Adhesive Systems	Time	CHX	p-Value
SB	Immediate	(–)	0.000
		(+)	0.000
	Aged	(–)	0.122*
		(+)	0.000
SE	Immediate	(–)	0.000
		(+)	0.000
	Aged	(–)	0.078*
		(+)	0.000
S ³	Immediate	(–)	0.000
		(+)	0.000
	Aged	(–)	0.116*
		(+)	0.000
PLP	Immediate	(–)	0.000
		(+)	0.000
	Aged	(–)	0.059*
		(+)	0.000

* No statistically significant difference, $p>0.05$.

yielding lower dentin bond strength compared with other groups ($p<0.05$). In aged CHX-pretreated groups, results were similar as with their *in vitro* counterparts (Table 5).

Figures 1 through 4 demonstrate representative scanning electron micrographs of the resin-infiltrated zone. Regardless of the type of adhesive, testing condition (ie, *in vivo* / *in vitro*), or CHX pretreatment, all specimens showed a well-defined, continuous hybrid layer that slightly varied in thickness along the resin-dentin interface.

DISCUSSION

While many studies have investigated the effects of CHX pretreatment on dentin bond strength, controversy still exists regarding whether CHX decreases immediate bond strength.^{12,16,19,21-24} In the present study, CHX treatment caused lower immediate dentin bond strength values in all test groups. In line with the present results, Campos and others²² observed that 2% CHX caused a decrease in immediate dentin bond strength values of self-etch adhesives, Clearfil SE Bond, and Clearfil S³ Bond. In the same study, however, CHX application had no influence on the immediate bond strength values of the tested etch-and-rinse adhesives. Other studies utilizing different self-etch or etch-and-rinse adhesives have reported that CHX has no effect on immediate bond strength values.^{12,19,23-26} In another

Table 5: Results for the Comparison of Means (MPa) of the Adhesive Systems Within the Levels of the Test Conditions, CHX Application, and Time

Adhesive Groups	In Vitro p-Value				In Vivo p-Value			
	CHX(-) Immediate	CHX(+) Immediate	CHX(-) Aged	CHX(+) Aged	CHX(-) Immediate	CHX(+) Immediate	CHX(-) Aged	CHX(+) Aged
SB vs SE	1.000*	0.336*	1.000*	1.000*	0.657*	1.000*	1.000*	1.000*
SB vs S ³	0.000	0.000	0.001	0.000	0.002	0.557*	0.000	0.000
SB vs PLP	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
SE vs S ³	0.000	0.000	0.000	0.000	0.259*	0.955*	0.000	0.000
SE vs PLP	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000
S ³ vs PLP	0.070*	0.000	0.217*	0.227*	0.735*	0.016	0.107*	0.077*

* No statistically significant difference, $p > 0.05$.

er study, CHX was found to preserve the bonding durability of an etch-and-rinse adhesive (Single Bond) but was unable to maintain a stable bond of a one-step self-etch adhesive system (GBond).²⁷ Because CHX generally inhibits MMPs without impairing dentin bond strength,²⁸ the benefits of CHX pretreatment might be expected over the course of time. Manfro and others²¹ demonstrated the effectiveness of CHX in preventing the degradation of the adhesive interface in primary dentin. In their study, they found no significant reduction in dentin bond strength in aged samples, while in the absence of CHX treatment, significant reductions were observed. The present results corroborate with those of previous studies reporting that CHX application has no adverse effect on aged dentin bond strength values. Further, the present study demonstrates that CHX treatment leads to higher aged dentin bond strength compared with non-CHX-treated groups. Therefore, the first null hypothesis should be rejected. It has been proposed that the use of 2% chlorhexidine before application of adhesive resins reduces the deterioration of hybrid layers following exposure to water.²⁹ In the present study, the higher bond strength values obtained in aged CHX-treated samples might be related with the inhibition effect of CHX on MMPs that had been activated by the acidity of the tested adhesives. As known, mild acids have a potential to activate MMPs, and particularly, pH values ranging from 2.3 to 5 are effective in activating gelatinases.³⁰ The self-etch adhesive used in this study has a pH value of 2.4 and is, thus, capable of enhancing dentin proteolytic activity without denaturation of the enzymes. The observation of increased aged bond strength after CHX pretreatment has been demonstrated previously, utilizing an etch-and-rinse and a self-etch adhesive.³¹

For the experimental groups that did not receive CHX-pretreatment, our results confirm previous studies that reported reductions in the dentin bond strength of different self-etch and etch-and-rinse adhesives after aging.³²⁻³⁷ For CHX-pretreated groups, however, establishing correlations between the present results and those of previous reports might be more complicated. It has been shown that CHX has no significant effect on bond strength stability, and that there was a decrease in bond strength over a six-month period of water storage.³⁷ Further, in an 18-month clinical study comparing the performance of an etch-and-rinse and self-etch adhesives in noncarious cervical lesions with and without CHX pretreatment,³⁸ the authors concluded that CHX did not influence the performance of the tested adhesives. Undoubtedly, clinical retention is

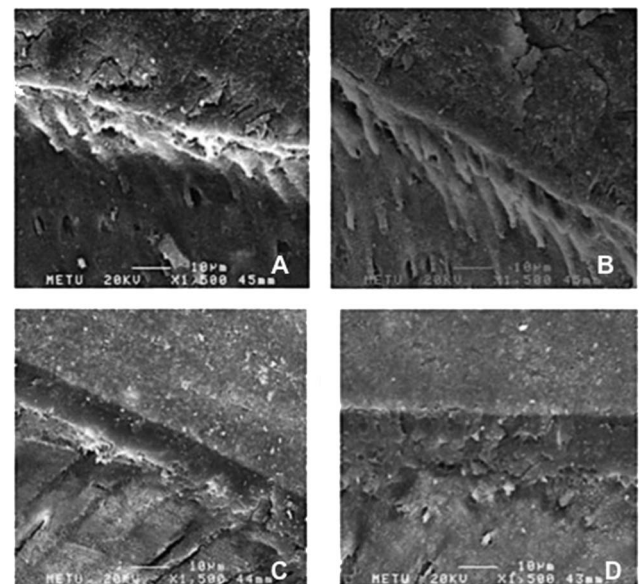


Figure 1. SEM images of composite-dentin interface with Adper Single Bond 2. (A): Immediate and CHX (+). (B): Immediate and CHX (-). (C): Aged and CHX (+). (D): Aged and CHX (-).

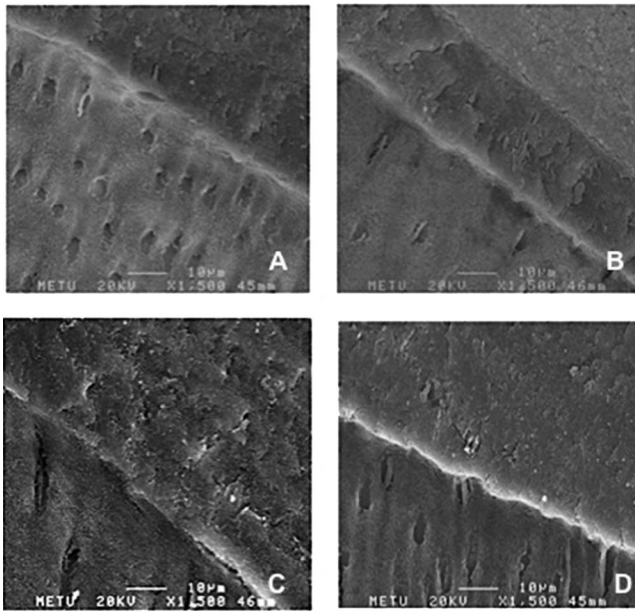


Figure 2. SEM images of composite-dentin interface with Clearfil SE Bond. (A): Immediate and CHX (+). (B): Immediate and CHX (-). (C): Aged and CHX (+). (D): Aged and CHX (-).

affected by many other factors, but those results should be considered when drawing conclusions.

Although clinical trials remain the ultimate instrument, preclinical screenings of materials are still important. However, it is still not fully understood whether there is a relationship between laboratory data and clinical outcomes and also whether the clinical performance is predictable in the lab. To date, only a few studies have compared the behavior of dental biomaterials under both clinical and laboratory conditions.^{11,37,39-41} The present results indicated differences in the bond strength values of CHX-pretreated groups, with higher bond strength values obtained in *in vitro* test results. This can be expected, particularly due to the insufficient simulation of oral conditions with thermocycling. Thermocycling is a common procedure to predict how a material would perform over time clinically. Although it is one of the most popular artificial aging methods, there is no evidence to indicate that thermocycling would create specimens equivalent to those fabricated *in vivo*. Patient-related factors constitute the difference between the laboratory and the clinical study. Comparing the bonding effectiveness of the etch-and-rinse adhesive to the two-step self-etch adhesive, it is evident that both adhesives bonded similarly under *in vivo* and *in vitro* conditions in non-CHX treated samples. Immediate bond strength values of one-step and all-in-one self-etch adhesives, Clearfil S³ Bond and Adper Prompt-L-Pop, were also similar. There-

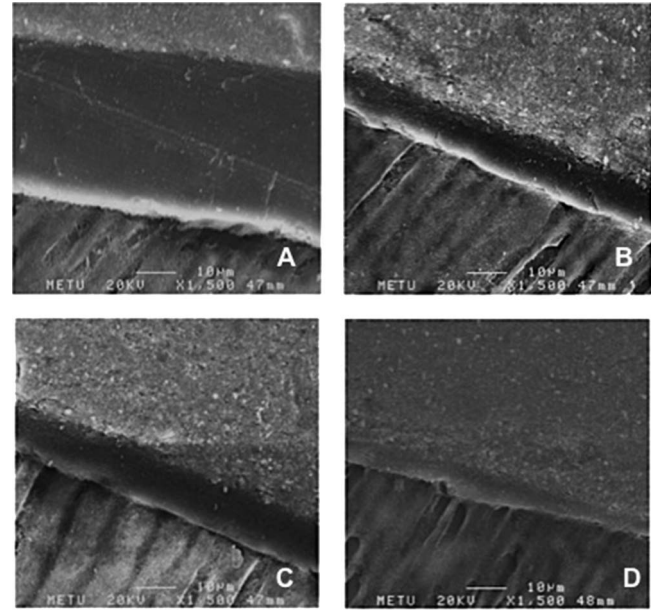


Figure 3. SEM images of composite-dentin interface with Clearfil S³. (A): Immediate and CHX (+). (B): Immediate and CHX (-). (C): Aged and CHX (+). (D): Aged and CHX (-).

fore, the second null hypothesis should be partly accepted.

In the present study, the bond strength values of Adper Single Bond and Clearfil SE Bond were significantly higher than the tested one-step self-etch adhesives. Several studies have shown that etch-and-rinse adhesives yield bonding values supe-

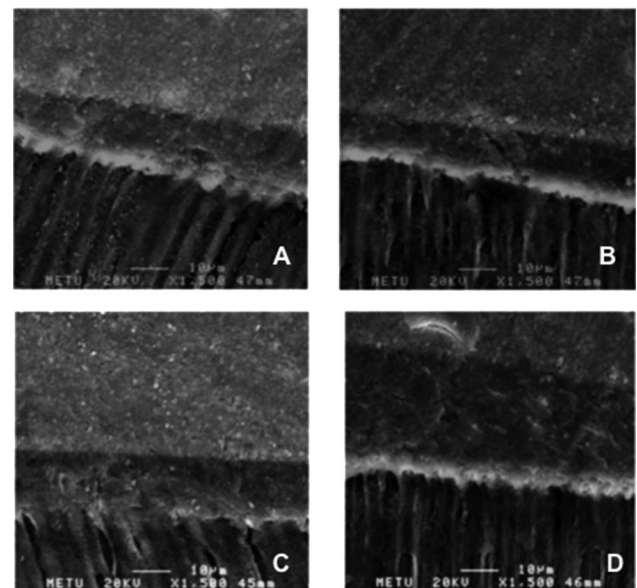


Figure 4. SEM images of composite-dentin interface with Prompt-L-Pop. (A): Immediate and CHX (+). (B): Immediate and CHX (-). (C): Aged and CHX (+). (D): Aged and CHX (-).

rior to those of one-step self-etch adhesives. Perdigao and others⁴² demonstrated that Adper Single Bond and Clearfil SE Bond showed similar bonding values that were also higher than one-step self-etch adhesives. Indeed, in several studies, Adper Single Bond and Clearfil SE Bond have shown significantly higher bond strength values compared with other different adhesives.^{34,43} The ethanol and HEMA content of Single Bond enables high wettability, whereas 10-MDP in Clearfil SE Bond binds to calcium salts that maintain a stable bonding interface.³⁴ Further, the relatively higher concentration of camphorquinone in Clearfil SE Bond improves its polymerization rate.⁴⁴

In the present study, the lowest bond strength values were obtained in aged one-step self-etch adhesives. Presumably, such levels of bond strength may result from the high acidity of these adhesives that degrades the collagen matrix stabilization.⁴⁵ It may be possible to speculate that additional factors, not possible to show with the present methodology, such as resin monomers with low conversion rate⁴⁴ and occurrence of water trees,^{34,45} may have contributed to these results. As bond strength values differ according to the tested adhesive systems, the third null hypothesis should also be rejected.

Adhesive and mixed failure types have been observed in a majority of microtensile bond strength studies, irrespective of CHX pretreatment.^{20-22,31,34} Osorio and others³⁴ reported that while mixed failure types were observed after immediate bond strength testing, adhesive failures were prominent in aged groups subjected to debonding forces. In the present study, predominance of adhesive/mixed failure was observed in all groups with a lack of statistical significance. In resin-dentin interface evaluation by SEM, the hybrid layer was found to be intact and mostly continuous in all tested samples.

Additional long-term studies might be necessary to determine the long-term effects of CHX on the bond strengths of different adhesive systems.

CONCLUSIONS

Within the limitations of this study, the following conclusions were drawn:

- 1) The immediate bond strength values of all tested adhesives were significantly higher than “aged” ones.
- 2) The application of CHX caused lower immediate and higher aged bond strength values.

Regulatory Statement

This study was conducted in accordance with all of the provisions of the local human subjects oversight committee guidelines and policies. The approval code for this study is LUT 09/72-21. This study was conducted at Hacettepe University.

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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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