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Effect of Chlorhexidine and/or Ethanol Pre-bonding Treatment on the Shear Bond Strength of Resin Composite to Dentin

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ABSTRACT

Aim of the study: *The aim of the study is to evaluate the effect of chlorhexidine and/or ethanol pre-bonding treatment on the shear bond strength of resin composite to dentin using etch and rinse adhesive system after 24 hours, 3 months and 6 months of aging.* ***Materials and methods:*** *Dentin surface of premolars were etched and randomly assigned into 4 groups (n=24). In Group A, dentin surfaces were bonded according to the manufacturer's instruction. In Group B and C, dentin surfaces were treated with 2% CHX or 100% ethanol for 60 sec respectively. In Group D, dentin surfaces were treated with 2% CHX for 60 sec followed by 100% ethanol for another 60 sec. The adhesive resin was then applied and light cured according to the manufacturer's instruction. Resin composite build ups were made above the dentin surface for shear bond strength test after 24 hours, 3 months and 6 months' storage period in normal saline. Bond strength data were analyzed using one-way ANOVA and Tukey's test. Mode of failure was also analyzed whether adhesive, cohesive or mixed between composite and dentin.* ***Results:*** *Significant differences were found for the 2 factors: 'storage period' ($p<0.05$) and 'adhesive protocol' ($p<0.05$) on bond strength. Numbers of mixed failure were higher when dentin surfaces were treated with 2% CHX and/or 100% ethanol.* ***Conclusion:*** *Dentin surface treatment with 2% CHX and/or 100% ethanol was effective in reducing the deterioration in shear bond strength of composite to dentin up to 6 months of storage.*

Keywords: Shear bond strength, Ethanol, CHX

INTRODUCTION

Composite is the material of choice in direct esthetic dentistry because of its high aesthetic quality that can mimic numerous shades and in different translucencies, conservative tooth preparation, and reinforcement of remaining tooth structure due to adhesion [1]. Despite the advancement that eliminate many of composite disadvantages; composite still has some shortcomings like polymerization shrinkage, technique sensitivity and lack of durable bond [2].

In adhesive dentinal bonding agents with etching and rinse technique, the minerals of dentin are removed leaving the organic matrix exposed which should be infiltrated completely by resin to form the hybrid layer after polymerization. Hybrid layer anchors firmly to dentin and adheres to the subsequent layer of composite [3].

In reality, resin monomer doesn't infiltrate the exposed organic matrix completely leaving the deepest part of the demineralized matrix partially or totally exposed and was subjected to nanoleakage later [4]. Enzymatic degradation of the organic matrix by matrix metalloproteinases (MMPs) together with the hydrolytic degradation of the hydrophilic resin components in the hybrid layer leads to the deterioration of the hybrid layer and the failure of the composite restoration later [5].

Many strategies were suggested to counteract the degradation in the hybrid layer to improve the lifespan of the restorations. One of these strategies was the use of different materials that act as MMP inhibitors like chlorhexidine, EDTA (ethylenediamine tetraacetic acid), Galardin, tetracycline and green tea extract [6]. The study of Tekce, et al., showed that the use of chlorhexidine as an intermediate agent between etching and bonding were able to reduce the bond strength reduction of the composite to dentin using micro tensile test after one year of storage [7].

Another strategy focuses on improving the infiltration of resin monomer into demineralized dentin for coating exposed collagen fibrils more efficiently thus reducing the nanoleakage and the hydrolysis of the hybrid layer, an example for this strategy is the use of ethanol after dentin demineralization. Ethanol is a solvent for hydrophilic and hydrophobic resin monomers that are able to expel water remnant from dentin and keeping collagen network distended to provide an easier path for resin infiltration [8,9].

MATERIALS AND METHODS

Sample Collection and Sectioning

Total 96 sound human maxillary premolar teeth were extracted for orthodontic demand with age range between 14-30 years. Teeth collected for the study were crack-free and sound when examined by a dental microscope (5X). The teeth were cleaned with a rubber cup and pumice and then washed with distilled water [10]. Teeth were stored in thymol solution (0.1%) for about 2-months till the time of the study to prevent bacterial and fungal growth [11]. The occlusal surface of each teeth was cut 1 mm below the mesial pit to expose a flat dentin surface using an electric diamond saw (Gamberini, Italy) under running water.

Adhesive Protocols

The exposed dentin surface was etched with 37% phosphoric acid (Alpha dent, USA) for 15 seconds and rinsed with water for 15 seconds, excess water was removed by a sponge leaving the surface visibly moist [12]. The teeth were then divided into 4 main groups of 24 teeth as follows:

Group A (control): Teeth belong to this group were coated with Single Bond Universal using disposable applicator with rubbing action for 20 seconds followed by gentle air blow for about 5 seconds with a triple syringe until it moved no longer and the solvent was completely evaporated [12,13].

Adhesive agent was then light-cured with LED light curing device (SDI, Australia) for 10 seconds according to the manufacturer's instruction. A specially designed handle was used to direct the light 2 mm away from the dentin surface in a vertical direction. The radiometer was used to measure power intensity before using the light curing device which was 600 mW/cm^2 [14].

Group B (CHX): After acid etching, teeth were rewetted with 2% CHX for 60 seconds using rubbing action. Teeth were then blot dried and the adhesive agent was applied and light cured as for the control group [14].

Group C (EWB): After acid etching, teeth were rewetted with 100% ethanol for 60 seconds using rubbing action [15]. Teeth were then blot dried and the adhesive agent was applied as mentioned in the control group.

Group D (CHX and EWB): After acid etching, teeth were rewetted first with 2% CHX for 60 seconds using rubbing action, blot dried then rewetted with 100% ethanol for another 60 seconds, blot dried and then the adhesive agent was applied as for the control group.

Filtek Z350 resin composite (3M ESPE, USA) was used for composite build ups placed in one single increment of 2 mm thickness and light cured for 40 seconds using an LED light curing device (SDI, Australia). Each group was further subdivided into 3 subgroups (n=8), subgroups 1 were subjected to shear bond strength test after 24 hrs, subgroups 2, 3 after three and six months respectively. Samples of all groups were stored in dark containers filled with distilled water and incubated in an incubator at 37°C till the time of the test.

Shear Bond Strength Testing

Shear bond strength of composite to dentin was tested using a universal testing machine (Tinius Olsen, Germany) at a crosshead speed of 1 mm/min. The acrylic blocks were fixed to the lower jaw of the testing machine in such a way that the chisel rod was held perpendicular to the composite tooth interface from the buccal aspect and the test continued until failure [16].

Mode of Failure

After shear bond strength testing, the tested samples were checked by the naked eye for any composite remnants [17]. Findings were supported by dying the samples using methylene blue dye and taking photos for the samples using a DSLR camera with macro-lens. Photos were collected and analyzed accurately by image software in the computer (JPG viewer). Teeth were analyzed to define whether the failure was adhesive, mixed (partially adhesive/partially cohesive) or cohesive failure within composite or dentin.

Statistical Analysis

The bond strength data were analyzed using a statistical package (IBM SPSS Statistics 20, Armonk, NY, USA). The data were analyzed with one-way ANOVA for the storage period and the adhesive protocol. Post-hoc multiple comparisons were carried out using Tukey's test with statistical significance set at ($p \leq 0.05$).

RESULTS

Shear Bond Strength

Table 1 shows the means and the standard deviations of the shear bond strength of the test groups. None of the samples failed before the test, results showed that all of the groups were affected by the storage time as shear bond strength values reduced over time.

Table 1 Mean and standard deviation of shear bond strength in (MPa)

Storage period	Groups	N	Mean	± S.D.	Min.	Max.
Immediate	A1	8	8.384	1.343	6.37	11.03
	B1	8	8.425	1.157	6.10	9.95
	C1	8	8.660	0.960	7.50	10.74
	D1	8	8.815	1.530	6.74	10.62
After 3 months	A2	8	7.151	0.903	5.71	8.63
	B2	8	8.006	1.570	6.28	10.85
	C2	8	7.707	1.562	6.30	10.95
	D2	8	8.202	0.714	7.11	9.44
After 6 months	A3	8	5.885	0.623	5.11	6.9
	B3	8	7.429	0.777	6.45	8.86
	C3	8	7.431	1.080	6.19	9.07
	D3	8	7.965	0.735	6.79	9.23

Effect of Storage Period

To see whether the storage period has an impact on the mean value of SBS for all groups, one-way ANOVA was used as seen in Table 2.

Table 2 F-test and p-value for all subgroups

Groups	Storage period	Comparison (d.f.=23)	
		F-test	p-value
A	A1	12.457	0.000 (HS)
	A2		
	A3		
B	B1	1.362	0.278 (NS)
	B2		
	B3		
C	C1	2.202	0.136 (NS)
	C2		
	C3		
D	D1	1.362	0.278 (NS)
	D2		
	D3		

No significant differences were found among the different subgroups of Group B, C, and D except for Group A as highly significant difference was found among its subgroups. Tukey-test was used as shown in Table 3 to find the subgroup responsible for the high difference.

Table 3 Tukey's HSD for A subgroups (control)

Storage period		Mean difference	p-value
A1	A2	1.232	0.056
	A3	2.498	0.000*
A2	A3	1.266	0.049*
*Significant ($p<0.05$) by Tukey-test			

No significant difference was found after 3 months when compared immediately. A significant difference was found between the 3 months and 6 months period and a highly significant difference was found between the immediate and 6 months period.

Effect of Adhesive Protocol

To examine the effect of different adhesive protocols, one-way ANOVA test was also used as shown in Table 4.

Table 4 The effect of different adhesive protocols

Durations	Groups	Comparison (d.f.=31)	
		F-test	p-value
Immediate	A1	0.206	0.891
	B1		
	C1		
	D1		
After 3 months	A2	1.007	0.375
	B2		
	C2		
	D2		
After 6 months	A3	9.563	0.000*
	B3		
	C3		
	D3		
*Significant ($p<0.05$) by one-way ANOVA			

A highly significant difference was found among subgroups only after 6 months and the non-significant difference was found among subgroups immediately and after 3 months, Tukey-test was used for further analysis as shown in Table 5.

Table 5 Tukey's HSD test after ANOVA test

Duration	Groups		Mean Difference	p-value
After 6 months	A3	B3	-1.543	0.004*
		C3	-1.546	0.004*
		D3	-2.08	0.000*
	B3	C3	-0.002	1.000
		D3	-0.536	0.567
	C3	D3	-0.533	0.571
*Significant ($p<0.05$) by Tukey-test				

A highly significant difference was found between the control and other groups and non-significant difference was found among other experimental groups.

Mode of Failure

After careful examination of the photos of the samples after testing, it had been shown that failures fallen in 1 of 2 categories:

- The adhesive failure that occurred between composite and dentin
- The mixed failure that involved failure within the composite and adhesive layer

However, no sample showed any failure within dentin as shown in Table 6.

Table 6 Modes of failure of the different groups

Groups	24 hours		3 months		6 months	
	Mixed	Adhesive	Mixed	Adhesive	Mixed	Adhesive
A	6	2	5	3	3	5
B	7	1	7	1	5	3
C	6	2	5	3	4	4
D	8	0	7	1	6	2

DISCUSSION

Effect of Storage Period

Control subgroups: The results of this study revealed that there was no statistically significant difference in SBS means between subgroups A1 and A2. This result can be explained by the action of MMPs in the 3 months which may be insufficient for producing significant bond strength reduction and this finding is supported by De Munk, et al., study who found non-significant difference between samples tested immediately and after 3 months of storage using microtensile bond strength test [18].

However, after 6 months of aging, statistically significant and highly significant differences were found as compared with the control group which indicates the continuous reduction of bond strength and loss of durability over time.

The loss of SBS could be due to the following:

- Human dentin contains collagenase (MMP-8), gelatinase (MMP-2) and (MMP-9), which are zinc-calcium endopeptidase trapped within the mineralized dentin matrix. When dentin is etched, these peptidases are activated allowing slow degradation of the unprotected collagen fibrils at the bottom of the hybrid layer [19,20]
- Inability to expel water completely from the expanded collagen fibrils may result in the breakdown of uncovered collagen fibrils [21]. Water is considered as an obligatory requirement for the action of MMPs, as no loss of bond strength over time was observed if mineral oil was used as a storage medium instead of water. Water is used in etch and rinse the adhesive system to remove phosphoric acid and keeping collagen fibrils distended for resin [20,22]
- Plasticization of adhesive which may happen with time because of water sorption by the hydrophilic resins in the hybrid layer which cause hydrolytic degradation of unreacted adhesive resin with reduction of bond strength over time. After hydrolysis, resin elutes through nano-channels exposing collagen fibrils and enhances the loss of bond durability over time [23]

These results are supported by the studies of Munoz, et al., and Nagi who found a significant difference between the control samples tested immediately and after 6 months using microtensile bond strength test [13,24].

Chlorhexidine subgroups: Comparing B subgroups, no statistically significant differences were found among them. The most reasonable explanation for this finding would be the ability of CHX to inhibit matrix metalloproteinase firstly by chelating calcium and zinc ions responsible for MMPs activation resulting in decreased degradation of the collagen fibrils over time and secondly by protein denaturation at higher concentration [25,26].

This result is supported by the studies of Carrilho, et al., Loguercio, et al., and Loguercio, et al., who used micro-tensile bond strength tests of the composite to dentin and found the non-significant difference between samples tested immediately and after the storage period [26-28]. The study of Shafiei, et al., was also supporting this study by using push-out bond strength test on fiber posts cemented to root dentin using total-etch technique with CHX after 2 years of storage, when comparing the control group with group treated with CHX; a statistically significant higher reduction in bond strength could be seen in the control group [29].

The *in vivo* study of Brackett, et al., partially supports the result of this study [30]. The transmission electron microscope was used to examine the degradation of the hybrid layer after 6 months of retention inside the patient's mouth. A slight degradation in the hybrid layer was observed in the control group with no degradation in the CHX group. However, the microtensile bond strength was also tested and no significant difference was found between the groups and this could be attributed to the different environments of these 2 studies.

Although the non-significant difference was found among subgroups, subgroup B3 was lower than B2 and the latter was lower than B1 in mean SBS values, and this finding could be explained by that collagenolytic degradation was reduced but did not stop completely to prevent any loss in bond strength. Also water can leach out uncured and hydrophilic monomer in adhesive systems resulting in loss of bond strength over time as modern adhesive systems contain a high percentage of hydrophilic monomers to enable bonding to wet dentin substrate, but this may produce permeable unstable resin matrices that are liable to water sorption, resin leaching, and hydrolysis over time [16,31,32].

Ethanol subgroups: Concerning Group C, results showed no statistically significant difference among its subgroups. This may indicate the ability of ethanol to preserve bond strength and improve durability. Ethanol can expel water from dentin providing a better medium for resin infiltration and enveloping more exposed collagen fibrils and preventing their degradation by MMPs [33,34], it also creates a more hydrophobic environment reducing water sorption over time which is a key factor in bond degradation [31,32,35].

This finding is supported by the studies of Sadek, et al., Ekambaram, et al., Simoes et al., Venigalla et al., who studied the microtensile bond strength of ethanol wet bonding in different periods of time [11,15,34,36]. And found a significant difference between the control group and the group treated with ethanol wet bonding.

However, this finding disagrees with the results of Ayar who found no statistically significant difference between ethanol and control group after 6 months of storage [37]. The reason behind this contrary in results could be attributed to the use of bovine incisors as a substrate for bonding which differs from human teeth and does not simulate natural situation.

Even though non-significant, time-dependent reduction among subgroups could be seen when looking at the results this could be explained by the same causes mentioned for the CHX groups.

Ethanol and chlorhexidine groups: Comparing the 3 subgroups of Group D, it showed preservation of bond strength over time. The reason for this finding must be clear as this technique might combine the positives of CHX and ethanol, as ethanol expel water thus improving resin infiltration and creates a more hydrophobic hybrid layer which reduces water sorption [31-35], while CHX act as an MMP inhibitor reducing hydrolysis of collagen fibrils thus improving the durability of the bond [18,25,30,37].

This result is supported by the study of Ekambaram, et al., [15]. Another study by Manso, et al., used two types of bonds, excite (Ivoclar Vivadent) and all bond 3 (Bisco) and their effect on microtensile bond strength when used with CHX and ethanol [17]. Adhesion with excite stabilized the bond after 15 months while all bond 3 did not, the reason behind that may be the higher ethanol content of all bond than excite (ethanol content for AB3 49%, Excite 2%), this high content may interfere with the bonding procedure by diluting resin excessively and preventing the formation of good quality hybrid layer.

Effect of Adhesive Procedure on Shear Bond Strength

Immediate shear bond strength: In this study, a statistically non-significant difference was found between the 4 groups tested immediately, and this means that neither positive nor negative influence of the different procedures could be seen when compared to control group and this agree with Khalil and Al-Shamma study and was supported by the studies of Loguercio, et al., and Manso, et al., [14,16,26]. It is also supported by the study of Guimaraes, et al., which was a microtensile bond strength test study for ethanol assessment and no significant difference was found between the control and ethanol groups [38].

Shear bond strength after three months: Results showed the non-significant difference between the 4 groups after 3 months of storage and this can be explained as mentioned previously that 3 months' period may be insufficient for the occurrence of a severe drop in bond strength allowing significant difference among groups. This finding is supported by the study of De Munck, et al., who found the non-significant difference between control and chlorhexidine group after 3 months using microtensile strength tests [18]. The results of Khalil and Al-Shamma disagree with this study as they found a significant difference between control and chlorhexidine group after 3 months and this could be attributed to the different adhesive agents and composite materials used in these two studies [14].

Shear bond strength after six months: When comparing the 4 groups after 6 months, a highly significant difference was found between the control and the other groups in mean SBS.

Control Group vs CHX Group

The control group showed a highly significant lower SBS after 6 months when compared with CHX group and this may reveal the role of CHX as MMPs inhibitor to prevent collagen degradation thus improving the durability of bond strength, this finding is supported by the studies of Brackett, et al., Carrilho, et al., Loguercio, et al., Boruziniat, et al., Loguercio, et al., and Shafiei, et al., [16,26-30].

Control Group vs Ethanol Group

The control group also showed a highly significant lower SBS than the ethanol group after 6 months which indicates the preservation of bond strength over time in the ethanol group. It can be explained as mentioned previously that ethanol acts as a solvent for both hydrophilic and hydrophobic resins found in the adhesive systems thus improving resin infiltration and coating the exposed collagen fibrils protecting them from MMPs activity and improving the bond durability [8,39]. This result is supported by Sadek, et al., Ekambaram, et al., Simoes, et al., and Venigalla, et al., [11,15,34,36].

Control Group vs CHX and Ethanol (Mixed)

A highly significant difference was found between the control and the mixed group after 6 months due to the high drop in the control group and this can be explained by the anti-degradation properties related to CHX and ethanol as mentioned previously, this finding is supported by the study of Ekambaram, et al., [15].

CHX Group vs Ethanol Group

The non-significant difference could be seen between these 2 groups after 6 months of storage. Even though ethanol and CHX act by different mechanisms to protect the hybrid layer, results showed that both of them are equally active after 6 months. This finding is supported by the study of Ekambaram, et al., [15].

In contrast, Sadek, et al., found a significant difference between ethanol and CHX [34]. The microtensile bond strength results were higher for the ethanol group after 18 months of storage. The reason behind that could be due to the use of an experimental hydrophobic adhesive resin with the ethanol group which creates a better hydrophobic environment reducing water sorption and bond degradation upon storage.

CHX and Ethanol Groups vs Mixed Group

No significant difference was found between CHX and a mixed group from one side and also no significant difference was found between ethanol and mixed groups from the other side. This finding may indicate the poor synergistic effect between CHX and ethanol when mixed together to produce a more durable bond with a significant difference. This finding is supported by the study of Sadek, et al., as they applied ethanol after CHX application in one group and no significant difference could be seen from the group with the sole ethanol application [34].

The study of Ekambaram, et al., is also supporting this study as they found a non-significant difference when they add CHX to ethanol and compare it to the ethanol group only [15].

Mode of Failure

The mode of failure found in this *in vitro* study was either adhesive or mixed failure with no cohesive failure in dentin or composite. The majority of failures were the mixed type of failure and this can be explained as no degradation in the hybrid layer occurred in this short period, which results in higher SBS values and failure was partially in composite and partially in the hybrid layer. The highest subgroup with mixed failures was subgroup D1 while other subgroups showed less mixed failures reflecting lower SBS values. This finding agrees with Khalil and Al-Shamma [14].

More adhesive failures could be seen after 3 months indicating the continuous degradation seen in most of the groups. Subgroup B2 an D3 were higher than A2 and C2 in numbers of cohesive failures and this finding also agrees with Khalil and Al-Shamma [14].

After 6 months, all of the 4 groups showed an increase in numbers of adhesive failures and this may indicate the degradation of the hybrid layer which became the weakest chain where the fracture occurred. The subgroup with the highest number of adhesive failure was the control subgroup A3 and this coincides with the fact that this group was the lowest in SBS mean value after 6 months. The lowest group with adhesive failure was group D3 and this coincides with the finding that this group showed the highest mean value of SBS.

CONCLUSION AND RECOMMENDATION

Within the limitations of this study, the following conclusions could be stated:

- At 24 hours and after 3 months, there is no significant difference in shear bond strength of composite to dentin between the control group and all experimental groups in which dentin surface was treated with 2% CHX and/or 100% ethanol
- After 6 months, deterioration of the shear bond strength of composite to dentin was seen in the control group
- After 6 months, dentin surface treatment with 2% CHX and/or 100% ethanol for 60 sec after acid etching and prior to adhesive application was effective in preventing the deterioration in shear bond strength of composite to dentin
- No statistically significant difference was seen between the use of 2% CHX alone, 100% ethanol alone or their successive application even though the latter showed a higher mean value of shear bond strength of composite to dentin

Depending on the results of the study, we recommend the combined use of 2% CHX and 100% ethanol after acid etching as a pre-adhesive application step or at least the use one of them to reduce the deterioration in bond strength and improve the longevity of the composite restoration.

DECLARATIONS

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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