

## Bond Strength of Adhesives to Dentin Surface Treated with Chlorhexidine

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### Abstract:

**Background:** The aim of this study was to evaluate the shear bond strength (SBS) of two types of adhesives (total and self-etch) to dentin surface treated with 2% of chlorhexidine (CHX) conditioner.

**Materials and Methods:** A total of 72 freshly extracted human sound premolar teeth were selected, washed and cleaned, and then divided randomly into three main groups; each tooth cusp was cut off until the first layer of dentin under the groove between the two cusps was exposed (superficial dentin). Then, 48 teeth were randomly selected to be used in Group I (control group) and Group II ( $n = 24$  each). The remaining 24 teeth were selected to be used in Group III which were cut off until the deep dentine between the first layer of exposed dentin and pulp horn was exposed. Then, adhesives were applied on the exposed dentin in Group I without application of CHX intermediate. In Group II, 2% CHX was applied to the superficial dentin surface for 1 min and washed away before application of adhesives. In Group III, 2% CHX was applied to deep dentin surface for 1 min and washed away before applying adhesives. Instron machine was used to assess the SBS. Data were analyzed using one-way ANOVA and Tukey's *post-hoc* test.

**Results:** The control group showed the higher mean SBS than the two groups as well as, bonding to superficial dentin in Group I and Group II showed higher mean bond strength when compared with deep dentin in both self-etch and total-etch groups. In general, self-etch showed higher bond strength than total etched adhesives.

**Conclusion:** Bond strength of adhesives without using CHX was the highest compared with using CHX as dentine surface conditioner. However, self-etch was stronger than total etch, and also adhesives were weakest in deep than superficial dentin.

**Key Words:** Adhesion, chlorhexidine, composite resin, deep dentin, dentin bonding, instron, self-etch, superficial dentin, total etch

### Introduction

During the past two decades, chemical and technical advances have contributed to increase resin-dentin bond strength. However, premature loss of bond strength is one of the

common problems that still affects adhesive restorations and markedly reduces their durability and longevity.<sup>1</sup>

The loss of bond strength has been attributed mainly to the degradation of the hybrid layer at the dentin–adhesive interface. This dentin–adhesive interface is an unstable layer and it deteriorates over time, which affect the bonding mechanism.<sup>2</sup>

In addition, to the heterogeneity of dental tissues, water plays an important role in obtaining adhesion. Water is believed to be related to the mechanisms of degradation at the bond interface and also with the reduction in mechanical properties of adhesive systems. The demineralized dentinal zone is not completely infiltrated by the resinous monomers<sup>3</sup> which would leave the exposed subadjacent collagenous fibrils susceptible to hydrolytic degradation, causing weakening of the hybrid layer and possibly decreasing adhesion.<sup>4,5</sup>

Chlorhexidine (CHX) is widely used in dentistry as an antimicrobial agent and for disinfection before placement of restorations. However, CHX solution is active against a wide range of microorganisms because it is bacteriostatic at low concentrations and bactericidal at higher concentrations.<sup>6</sup>

In addition to its antimicrobial effect, it was found that application of 2% CHX for 60 s inhibited the collagenolytic activity of dentin surface and found that it has an inhibitory action on matrix metalloproteinase (MMP) enzyme.<sup>7</sup>

As a result of the dentin mineralization process at the stage of dentinogenesis, these enzymes are retained in the extracellular matrix in a latent state but can be activated if the dentin is demineralized. Therefore, it is expected that the MMP will be released in the process of collagen exposure by acid etching and is related to the loss of bond strength over time.<sup>8</sup>

### Materials and Methods

#### Study design

This is a laboratory-based experimental study assessing bonding strength of two types of adhesives to dentin surface conditioned with and without CHX.

#### Sample size and teeth selection

A total of 72 freshly extracted human sound premolar teeth were used in this study. The teeth were washed under running water immediately after extraction, cleaned and stored in 2%

thymol solution until the experiment time. Then, the teeth were randomly divided into three main groups, each consists of 24 teeth.

### Specimen preparation

Each tooth cusp was cut with a low-speed diamond disk under water coolant.

Cutting was carried out until the first layer of dentin under the groove between the two cusps was exposed (superficial dentin). Then, 48 teeth were randomly selected to be used in Group I and Group II.

The remaining 24 teeth were selected to be used in Group III. In this group, deep dentin was exposed as follows; further cutting exposed superficial dentin until deep dentin between the first layer, and pulp horn (as shown on bucco-lingual or bucco-palatal direction) was exposed.

Dentin surfaces of all the samples were flattened using 600 grit silicon carbide papers to create a standardized smear layer.<sup>9</sup>

Silicon mold was used to fabricate composite cylinder (2 mm height and 1 mm diameter) to represent composite restoration adhering on the dentin surface using total- and self-etch to be exposed to Instron shear bond strength (SBS) (Figure 1).

### Bonding materials

The bonding materials used in this study are self-etch (3M ESPE Scotchbond universal SML Dental Self Etch Adhesive) adhesive and total-etch (3M ESPE Adper Single Bond Plus Adhesive) "conventional" system, which will be used according to manufacturer's instructions as shown in Table 1.

### The experimental groups

The three main groups were prepared and subdivided into six subgroups as follows:

#### Group I: Control (24 specimens)

In this group, the adhesive was applied on the exposed superficial dentin surface without using CHX, according to

manufacturer's instructions (Figure 2). Then, teeth were subdivided into two subgroups (Group IA and Group IB), 12 samples in each group. In Group IA, total-etch adhesive was used, while in Group IB, self-etch adhesive was used (Table 2). After adhesive application and composite application, composite cylinder was adhered on the tooth surface. After that, samples were stored in water for 24 h and then they were exposed to SBS test using Instron machine.

#### Group II: CHX on superficial dentin (24 specimens)

In this group, 2% CHX was applied to superficial dentin surface for 1 min and washed away. Then, teeth were subdivided into two subgroups (Group IIA and Group IIB), 12 samples in each group. In Group IIA, total-etch adhesive was used, while in Group IIB, self-etch adhesive was used (Table 2). After adhesive application and composite application, composite cylinder was adhered on the tooth surface. After that samples were stored in water for 24 h, they were exposed to SBS test using Instron machine.

#### Group III: CHX on deep dentin (24 specimens)

In this group, deep dentin surface was exposed as described above in teeth preparation step. After that, 2% CHX was applied to the dentin surface for 1 min and washed away, and then the adhesives were applied according to manufacturer's instruction. Then, the teeth were subdivided into two subgroups (Group IIIA and Group IIIB), 12 samples in each group. In Group IIIA, total-etch adhesive was used, while in Group IIIB, self-etch adhesive was used (Table 2). After adhesive application and composite application, composite cylinder was adhered on the tooth surface. After that, the samples were stored in water for 24 h, and then they were exposed to SBS test using Instron machine.

### Restoration placement procedure

A resin-based composite 3M ESPE Filtek Z250 was applied and cured for 40 s for all specimens after bonding procedures according to the divided groups. After the resin restoration, we used the Elipar™ S10 LED Light Curing unit (3M ESPE, Seefeld, Germany) at a power after light activation of 9600 MW/cm<sup>2</sup> with a halogen light source.

Table 1: Bonding materials used in the study with the manufacturer instructions.

Material/manufacturer	General composition	Manufacturer's instructions for use
Total etch: 3M ESPE Adper Single Bond Plus Adhesive	Acid: 37% phosphoric acid Bond: Acetone, bonding ormoocer dimethacrylate, acid-modified methacrylates, initiators, and stabilizers	<ul style="list-style-type: none"> <li>Dentin was etched for 10 s with 37% phosphoric acid, rinsed with water spray for 5 s</li> <li>Bond was applied with disposable brush, thinned with mild air for 2-3 s, and light cured for 20 s</li> </ul>
Self-etch: 3M ESPE scotch bond universal SML Dental self-etch adhesive	Methacrylated phosphoric esters Bis-GMA Initiators based on camphorquinone Stabilizers Water 2-Hydroxyethyl methacrylate (HEMA) Polyalkenoic acid Stabilizers	<ul style="list-style-type: none"> <li>Apply bond with a brush and air-thin</li> <li>Light cure for 20 s</li> </ul>

### Specimen preparation for bond strength test

After adhesive application, polyethylene micro-bore tubing of 2.0 mm in height with an internal diameter of 1.0 mm was placed on the treated surfaces and applied the composite resin (Figures 3 and 4).

Using light activation after composite resin was carefully inserted into the tubing lumens and irradiated for 40 s according to the manufacturer's instructions. After curing, the tube around the composite cylinders was removed by gently cutting the tube into two hemi cylinders using a featheredge blade.

### SBS

For shear test, each tooth segment was attached to the testing apparatus with a cyanoacrylate adhesive and was tested in universal testing machine (Instron machine). With the load cell projection and the resin cylinder making contact together with the lower half circle and touching the tooth surface, force was applied at a crosshead speed of 0.5 mm/min until failure occurred. Care was taken to keep the composite cylinder in line with the center of the load cell and to keep the wire loop parallel to the load cell movement direction and to the bonded surface to maintain a shear stress orientation at the bonding interface. The maximum loads (Mpa) at the time of failure were recorded for every sample.

### Bonding failure mode testing

Each bonding interface was tested under a light microscope (LM) (Nikon digital camera DX1200F) for bonding failure mode at a magnification of  $10 \times 40$ .

### Data analysis

Data collected were analyzed using descriptive statistics to describe the frequency and distributions of the evaluated criteria. The data were analyzed using a commercially available statistical software package (SPSS 21.0, SPSS Inc.).

### Results

This study evaluated two types of bonding agents with and without CHX conditioner using Instron machine to assess bond strength. It was found that when comparing the three groups (Group 1 with Group 2 and Group 3), the bond strength in Group 1 without CHX conditioner was found highly statistically significant ( $P = 0.000$ ).

### SBS

Quantitative data obtained from the testing machine revealed a statistically significant difference in the mean SBS values (MPa) in self-etch bonding system than total-etch system in all groups.

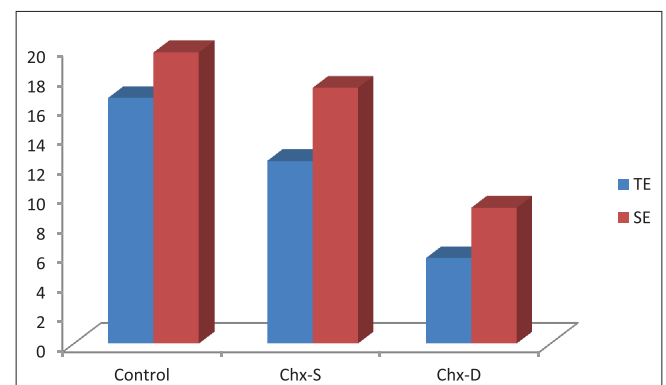
### Univariate statistics

In control group (Group I) where no CHX was used, it showed higher mean bond strength than the two experimental groups

**Table 2: Experimental groups and number of specimens in each group.**

Groups	No.	Subgroups	No.
Group I control group without application of CHX at superficial dentin	24	Subgroup (A) total-etch adhesive	12
		Subgroup (B) self-etch adhesive	12
Group II after application of CHX at superficial dentin	24	Subgroup (A) total-etch adhesive	12
		Subgroup (B) self-etch adhesive	12
Group III after application of CHX at deep dentin	24	Subgroup (A) total-etch adhesive	12
		Subgroup (B) self-etch adhesive	12

CHX: Chlorhexidine



**Graph 1:** Mean shear bond strength values for all study groups in MPa.

(Group II CHX superficial dentin and Group III CHX deep dentin) where CHX was used before bonding procedure (Graph 1).

Bonding to superficial dentin in Group 1 and Group 2 showed higher mean SBS when compared to deep dentin in both self-etch and total-etch groups. The mean bond strength values for all the study groups are shown in Figure 5.

Table 3 shows mean bond strength in all the groups. In Group 2 where CHX was applied to superficial dentin, the mean bond strength was higher in self-etch sub-group (17.3 MPa) than total-etch sub-group (12.3 MPa). In Group 3 where CHX was applied in deep dentin, the mean bond strength was 9.2 Mpa for self-etch sub-group and 5.7 MPa for total-etch sub-group. The total mean bond strength for all the groups was 13.48 MPa.

From the results of this study, the mean SBS (MPa) and their standard deviations in Group 1 (control) of total-etch was  $16.609 \pm 12.494$  and self-etch was  $19.690 \pm 8.129$ , in Group 2 (CHX in superficial dentin), total-etch was  $12.348 \pm 5.506$  and self-etch was  $17.293 \pm 3.398$ , and in Group 3 (CHX in deep dentin), total-etch was  $5.777 \pm 2.143$  and self-etch was  $9.184 \pm 4.062$ . The maximum mean SBS was found in Group 1 (control) of self-etch sub-group ( $19.690 \pm 8.129$ ) as shown in Table 3.

Table 3: Mean and standard deviation of bond strength (MPa) in each group.

Group	Type	N	Mean	Standard deviation	Standard error mean
Control	TE	12	16.6095358	12.49493937	3.60697830
	SE	12	19.6908850	8.12999565	2.34692759
CHX superficial dentin	TE	12	12.3487700	5.50679959	1.58967611
	SE	12	17.2938892	3.39849825	0.98106194
CHX deep dentin	TE	12	5.7776033	2.41343324	0.69669817
	SE	12	9.1849450	4.06299181	1.17288471

TE: Total-etch, SE: Self-etch

The mean marginal SBS was highest in control group and self-etch sub-group and least in CHX applied on dentin deep surface (CHX-d) group using total-etch sub-group (Graph 2).

Mean SBS of control group was the highest ( $18.150 \pm 10.428$ ) followed by CHX-s group ( $14.821 \pm 5.138$ ) and CHX-d group ( $7.481 \pm 3.702$ ) as shown in Table 4 and Graph 3.

**Bivariate statistics**

Independent sample *t*-test showed a statistically significant difference between SBS and type ( $P = 0.05$ ). The mean SBS of self-etch type ( $15.389 \pm 7.097$ ) was more than the total-etch type ( $11.578 \pm 8.991$ ) as shown in Table 5.

**Multivariate analysis**

Analysis of variance (ANOVA) showed a statistically significant difference in the SBS between the groups ( $P = 0.000$ ) and between the types of bonding ( $P = 0.022$ ). The interaction effect was not statistically significant ( $P = 0.883$ ) as shown in Table 6.

Tukey's *post-hoc* analysis showed a statistically significant difference between control and CHX deep dentin groups ( $P = 0.000$ ) and CHX with superficial dentin and CHX deep dentin groups ( $P = 0.02$ ) (Tables 7 and 8).

Tukey's *post-hoc* analysis showed that in total-etch type, there is a statistically significant difference in the SBS between control and CHX deep dentin groups ( $P = 0.006$ ). In the self-etch type, there is a statistical significant difference in the SBS between control and CHX deep dentin groups ( $P = 0.000$ ) and CHX superficial dentin and CHX deep dentin groups ( $P = 0.003$ ).

**Mode of bond failure**

When studying the bonding interface under a light microscope, it was reported that the high mode of failure was adhesive (83%). In the first group (control) for total-etch, 10 specimens showed adhesive failure and 2 showed cohesive failure; for self-etch, the failure mode was adhesive in all samples ( $n = 12$ ). In the second group (superficial dentin) with CHX conditioning, failure mode in total-etch group was adhesive in 9 specimens and cohesive in 3 specimens, while for self-etch group, the failure mode was adhesive in 11 specimens

Table 4: Mean shear bond strength of the groups.

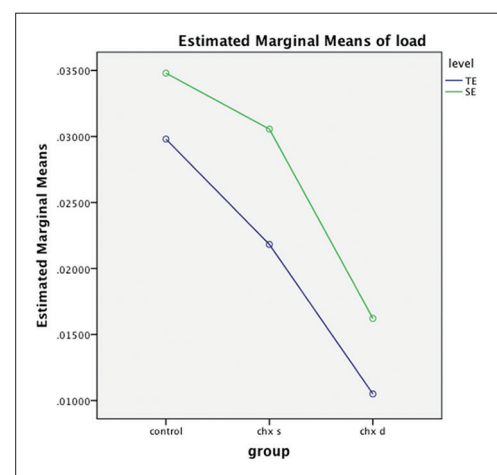
Group	Mean	N	Standard deviation
Control	18.1502104	24	10.42862283
CHX-s	14.8213296	24	5.13871145
CHX-d	7.4812742	24	3.70263156
Total	13.4842714	72	8.26866538

CHX-s: Chlorhexidine applied on superficial dentin, CHX-d: Chlorhexidine applied on deep dentin

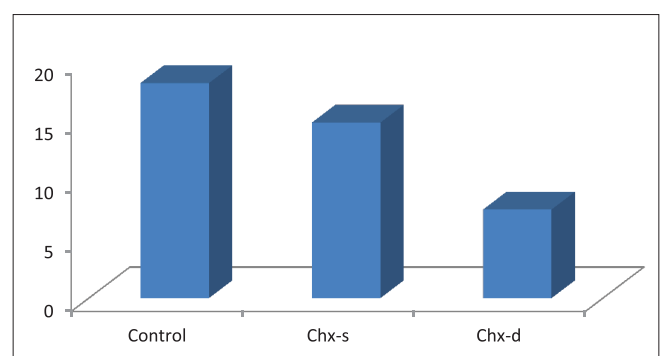
Table 5: Mean shear bond strength of adhesive type.

Type	N	Mean	Standard deviation	Standard error mean
TE	36	11.5786364	8.99152554	1.49858759
SE	36	15.3899064	7.09768796	1.18294799

TE: Total etch, SE: Self-etch



Graph 2: Mean marginal shear bond strength of groups and types of bonding.



Graph 3: Mean shear bond strength of the groups.

and cohesive in 1 specimen. In the third group (deep dentin) with CHX conditioning, failure mode in total-etch group was

Table 6: Analysis of variance between patients.

Tests of between-subjects effects					
Dependent variable: Compress stress load					
Source	Type III sum of squares	Df	Mean square	F	Significance
Corrected model	1703.626 <sup>a</sup>	5	340.725	7.137	0.000
Intercept	13091.441	1	13091.441	274.236	0.000
Group	1430.272	2	715.136	14.980	0.000
Type	261.464	1	261.464	5.477	0.022
Group*Type	11.889	2	5.945	0.125	0.883
Error	3150.703	66	47.738		
Total	17945.770	72			
Corrected total	4854.329	71			

<sup>a</sup>R<sup>2</sup>=0.351 (Adjusted R<sup>2</sup>=0.302)

Table 7: One-way analysis of variance between the groups.

Multiple comparisons								
Compress stress load								
Tukey's HSD								
(I) Group		(J) Group		Mean difference (I-J)	Standard error	Significance	95% Confidence interval	
							Lower bound	Upper bound
Dimension2	Control		CHX-s	3.3288808	2.03355197	0.237	-1.5421104	8.1998721
			CHX-d	10.6689363*	2.03355197	0.000	5.7979450	15.5399275
	CHX-s	Dimension3	Control	-3.3288808	2.03355197	0.237	-8.1998721	1.5421104
			CHX-d	7.3400554*	2.03355197	0.002	2.4690641	12.2110467
	CHX-d	Dimension3	Control	-10.6689363*	2.03355197	0.000	-15.5399275	-5.7979450
			CHX-s	-7.3400554*	2.03355197	0.002	-12.2110467	-2.4690641

Based on observed means.

The error term is mean square (error)=49.624.

\*The mean difference is significant at the 0.05 level. CHX-s: Chlorhexidine applied on superficial dentin, CHX-d: Chlorhexidine applied on deep dentin

Table 8: Two-way analysis of variance between the types and groups.

Multiple comparisons								
Dependent variable: load								
Type		(I) group	(J) group	Mean difference (I-J)	Std. error	Significant	95% Confidence interval	
							Lower bound	Upper bound
TE	Tukey's HSD	Control	CHX-s	0.00798333	0.00570591	0.353	-0.0060178	0.0219845
			CHX-d	0.01930333*	0.00570591	0.005	0.0053022	0.0333045
		CHX-s	Control	-0.00798333	0.00570591	0.353	-0.0219845	0.0060178
			CHX-d	0.01132000	0.00570591	0.132	-0.0026811	0.0253211
		CHX-d	Control	-0.01930333*	0.00570591	0.005	-0.0333045	-0.0053022
			CHX-s	-0.01132000	0.00570591	0.132	-0.0253211	0.0026811
SE	Tukey's HSD	Control	CHX-s	0.00423583	0.00404166	0.552	-0.0056816	0.0141532
			CHX-d	0.01857500*	0.00404166	0.000	0.0086576	0.0284924
		CHX-s	Control	-0.00423583	0.00404166	0.552	-0.0141532	0.0056816
			CHX-d	-0.01433917*	0.00404166	0.003	0.0044218	0.0242566
		CHX-d	Control	-0.01857500*	0.00404166	0.000	-0.0284924	-0.0086576
			CHX-s	-0.01433917*	0.00404166	0.003	-0.0242566	-0.0044218

\*The mean difference is significant at the 0.05 types. CHX-s: Chlorhexidine applied on superficial dentin, CHX-d: Chlorhexidine applied on deep dentin, TE: Total-etch, SE: Self-etch

Table 9: Mode of bond failure in each group.

Group	Control		Superficial CHX		Deep CHX	
	Adhesive	Cohesive	Adhesive	Cohesive	Adhesive	Cohesive
Total-etch (%)	10 (83)	2 (17)	9 (75)	3 (25)	11 (92)	1 (8)
Self-etch	12 (100)	0	11 (92)	1 (8)	10 (83)	2 (17)

CHX: Chlorhexidine

adhesive in 11 specimens and cohesive in 1 specimen, and for self-etch group, it was 10 adhesive and 2 cohesive (Figure 5 and Table 9).

**Discussion**

This study assessed the bonding strength of total-etch (3M ESPE Adper Single Bond Plus) and self-etch (3M ESPE

Scotchbond universal) adhesive systems with and without CHX conditioner.

The present study employed shear test to compare bond strength values of self-etched adhesive and total-etched adhesive systems. As it uses specimens with cross-sectional areas of reduced size, this test provides more uniform interfacial stress distribution, which in turn decreases the incidence of cohesive failures, both in dentin and resin.<sup>10</sup>

Adhesives were applied on superficial and deep dentin surfaces, after dentin surface conditioner with CHX. This was compared with control group where no CHX was used. The results of this laboratory-based study revealed that dentin SBS depends on adhesives with and without CHX conditioning.

This study investigated both the SBS using self-etched adhesive and total-etch adhesive in superficial and deep dentin with and without 2% CHX conditioning. In fact, all materials are biocompatible and have been approved for clinical use, thus making the results of this study clinically relevant.<sup>11</sup>

In this study, it was found that the mean SBS was higher in superficial dentin when compared with deep dentin in both



**Figure 1:** Specimens of each main group divided into two subgroups.

self-etch and total-etch groups. This is attributed to that deep dentin has wider dentinal tubules that occupy more surface area of dentin than the superficial dentin. Also, due to the high rate of moisture in the deep dentin, which makes the adhesion difficult. It was found a higher bond strength in self-etched adhesives than total etched adhesives. One of the advantages of self-etched adhesives is complete resin infiltration for the interfibrillar spaces, while the total-etch adhesive may not completely infiltrate and fill these spaces. This incomplete hybrid layer formation could be the reason behind the lower mean SBS values obtained in our study.<sup>12</sup>

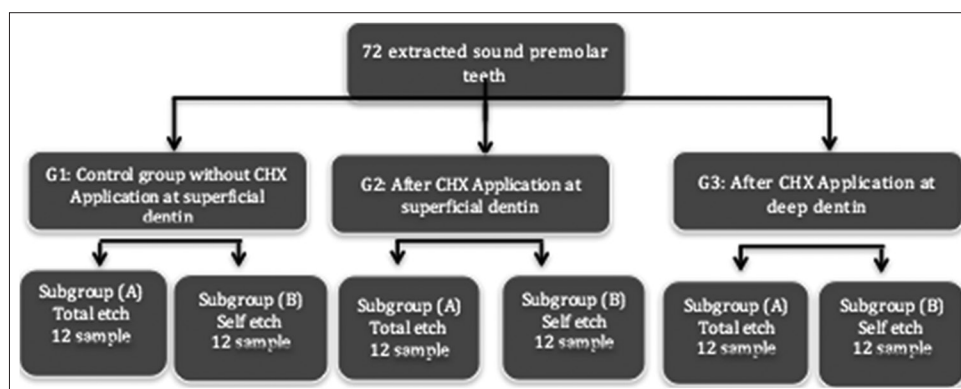
In fact, several studies revealed that self-etch system is stronger than total-etch when used on enamel and dentin. In contrast, other studies reported controversial findings where they advocated inferiority of self-etch systems.<sup>13-16</sup>

Therefore, our findings agree with that of Singla *et al.*<sup>17</sup> who reported that the self-etching adhesive systems produced even better bond strengths to both enamel and dentin than conventional total-etch systems, especially the “all-in-one” system, which produced the highest bond strength to enamel.

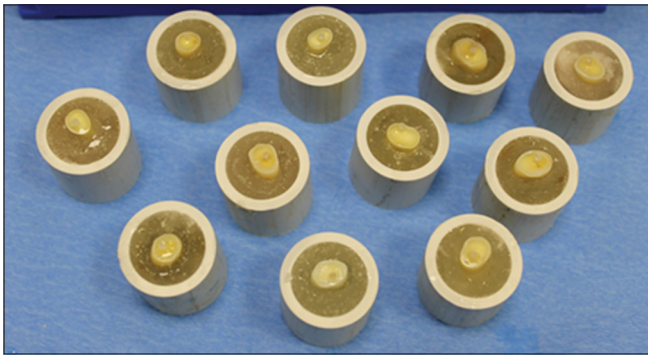
In addition, Villela-Rosa *et al.*<sup>18</sup> demonstrated the highest mean values of SBS of self-etching adhesive. It may be concluded that the SBS of dentin is dependent on material adhesive system, substrate depth, and adhesive/depth interaction, which is consistent with our findings.

The dental literature shows the association of cavity disinfectant application and bond strength values to be a contentious issue.<sup>19</sup> Data obtained in our study showed that mean SBS is higher in control group than CHX group, which indicates no improvement in the bonding effect of using CHX conditioning at the immediate loading. This could be due to the action of inhibitory effect of CHX.<sup>20</sup>

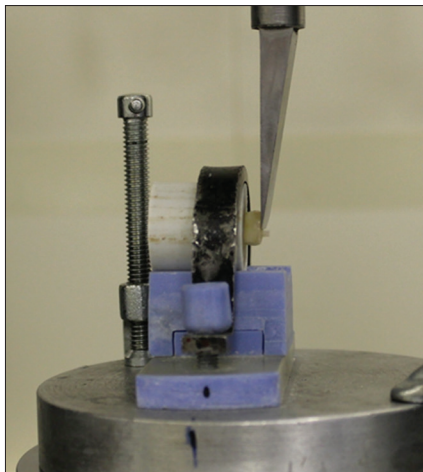
Our results agree with that of Mazzoni *et al.*<sup>21</sup> who stated that CHX inhibits bacterial to surface on dentinal tubules each other by coming with calcium for retention sites and thus prevent



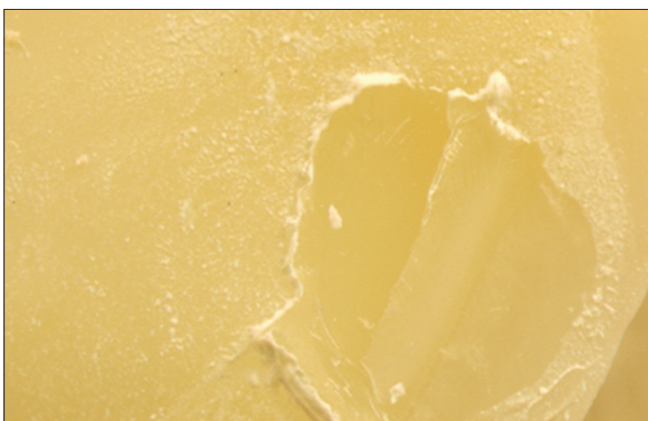
**Figure 2:** Flow chart of experimental design.



**Figure 3:** Samples ready for Instron machine testing.



**Figure 4:** Ready for shear bond strength by using Instron machine.



**Figure 5:** Example of adhesion failure mode.

the formation of calcium bridges between the bacteria and oral surfaces or between bacteria itself if we apply the CHX after acid etching stage. But not agree with bond strength when used CHX before adhesive the surface, our result are low bond strength with it.

Our results agree with that of Pashley *et al.*<sup>5</sup> who revealed the inhibitor effect of 2% CHX on collagenolytic activity of dentin surface and found that it has inhibitory action on MMP enzyme.

### Conclusion

Under the limitations of this study we conclude that:

- Self-etch bonding systems were stronger than total-etch systems.
- Bond strength of adhesives without using CHX was better than using
- CHX as dentine surface conditioner.
- Also, adhesives were weakest in deep dentin than superficial dentin.

### Recommendation

We recommend to use adhesives without using CHX as dentin conditioner and to use self-etch systems when possible than using total-etch systems.

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