Scholars Journal of Dental Sciences
Abbreviated Key Title: Sch J Dent Sci
ISSN 2394-4951 (Print) | ISSN 2394-496X (Online)
Journal homepage: https://saspublishers.com

OPEN ACCESS

In Vitro Study of Influence of Carisolve on Bond Strength of Composite with Dentine

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DOI: 10.36347/sjds.2022.v09i05.004 | **Received:** 29.05.2022 | **Accepted:** 27.06.2022 | **Published:** 30.06.2022

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Abstract **Original Research Article**

Aim of the Study: Chemomechanical caries removal using carisolv has been discussed as an alternative method to a conventional excavation system. The purpose of this in vitro study was to evaluate the influence of two caries removal techniques (conventional bur, and Chemomechanical removal/Carisolv™) on dentine shear bond strength (SBS) using SEM. ***Materials and Methods:*** The sample consisted of forty recently extracted permanent human molar divided into four groups. 30 Molars with coronal dentine caries extending approximately half-way through the dentine were randomly divided into three equal groups. Group 1: Caries was removed using Carisolv and the dentine surfaces of the sample was etched before applying composite bonding agent. Group 2: Caries was removed using carisolv without etching the dentine surface before applying bonding composite resin. Group 3: Caries was removed using a low-speed burs and hand excavation. Group 4: the Control Group consists of ten molars with Sound occlusal dentin surfaces. After preparing the sample they were subjected to thermo cycling for 500 cycles at 5-55°c with dowel time 15 seconds, and then they were tested in INSTRON device for SBS tests. Scanning electron microscopy was used to ascertain the kind of failure at the composite dentine interface. Data were analyzed by ANOVA and Bonferroni tests (P = 0.05). ***Results:*** Results showed that there was no different in bond strengths between Carisolv group and sound dentin group which were higher than the bond strength of mechanical group and Carisolv group without etching (P = 0.05). Failure at the level of restoration material was cohesive specially with sound dentine samples which have the higher bond strength. ***Conclusions:*** Chemomechanical caries using Carisolv enhanced the bond strength to dentine surface comparing with mechanical caries removal.

Keywords: Shear bond strength-SEM-Carisolv.

INTRODUCTION

Modern dentistry aims to treat the carious lesion conservatively, to preserve healthy dentin tissue during the excavation of caries [1]. The carious dentin consists of two layers: the severely affected outer layer, which is the most contaminated with bacteria, and the inner layer least affected [2].

Any effective technique for removing carious dentine should be able to stop the progression of the carious lesion and allow remineralization of the remaining dentin. Removal of partially demineralized dentin containing low numbers of bacteria is not necessary and may on the contrary, cause unnecessary removal of tooth tissue as well as possible exposure of the dental pulp [4].

Hence, the techniques of excavation dental caries received a great development, from rotary hand tools in (1728) to the use of rotating burs on turbine hand pieces in (1957), but because of the discomfort and pain that the patient feels during the excavation, the doctor is forced to use anesthesia to relieve the patient from pain during procedure. This prompted scientists to invent Chemo mechanical caries removal technique as an alternative solution to the mechanical excavation [5]. Which will give the chance to remove the severely decayed dentin, leaving the intact demineralized dentin for the opportunity of re-mineralizing.

This technique relies on a Carisolv™ material with the help of special excavators [6, 7]. Carisolv consists of two tubes: first tube contains a 0.95%

Sodium hypochloride liquid Naocl, and the second tube contains a gel of three amino acids (glutamic acid - lysine acid - leucine acid), also it contains methyl carboxy cellulose, sodium hydroxide and water [8, 9].

Sodium hypochloride has non-specific proteolytic effects [10], but when the gel and liquid mix in the syringe, the amino acids react with chlorine to form chloramines with high pH. Formation of chloramines reduces the reactions of chlorine without changing its function.

The three amino acids present are among the 20 acids that are medically used to build proteins, and they have different charges, and this allows their electrons to be attracted to different regions of the protein in the carious dentin [11]. Since the peptide chains of all proteins, including collagen, are made from small hydrophilic pieces (positive and negative charges) and uncharged hydrophobic pieces so each of the three amino acids attracts one of these small pieces electronically, causing effective force to the entire length of collagen fiber [12]. It is possible that chlorinated amino acids can destroy different types of electronic bonds in the fibrous structure and thus the chemical result is the breakdown of the damaged collagen present in the carious lesion. When the damaged collagen breaks down, it will be removed easily by Carisolv excavator without applying any pressure [11].

Since the shape and nature of the surface of the dentin remaining after carious excavation affects the bond strength of the adhesive materials [13], and because of the lack of information about the effect of chemo mechanical caries removal by Carisolv on the bonding forces of the composite, which in most cases is estimated by the value of the mechanical forces needed to generate a final separation between the bonded surfaces, which is usually calculated by measuring the Shear Bond Strength forces, so we carried out this study.

Aim of Study

A laboratory study to evaluate the bonding strength of the composite with the remaining dentin after excavation carious dentine by Carisolv with or without dentin etching, by applying shear bond tests and comparing the results with the mechanical excavation by using low speed hand piece and manual excavators and considering that the control group is the bonding strength with healthy dentin. The fracture surface caused by shear forces was studied under a scanning electron microscope to find out the location of the failure.

MATERIALS AND METHODS

The Research Sample

Forty freshly extracted permanent human molars were collected from the extraction department at the Faculty of Dentistry at Damascus University, for (surgical and prosthetic) purposes. They were cleaned and washed well, then placed in a sterile 5% chloramine solution, then kept in a saline solution at room temperature until use. It was later divided into 30 permanent human molars containing occlusal caries extending on the x-ray to the middle of the distance between the dentino-enamel junction and the pulp. This was subdivided into three groups equally. First group: consisted of 10 molars, the carious dentine in this group was removed by CarisolvTM (MediTeam-Sweden) which was applied for 30 seconds and swept away by the excavator (#2, #4) related to the system. The application of Carisolv was repeated and excavated until the gel applied became clear, and this was accompanied by hearing scratching sound of sound dentine by using the probe. Then the level of the surface was adjusted to the level of the cavity bottom by a diamond fissure bur applied to the edges of the cavity. After that Carisolv was applied for 3 minutes and washed again. The second group: consisted of 10 molars, the carious dentine was removed by the Carisolv similar to the first group but there was no etching before composite application in this group. The third group: consisted of 10 molars, the caries in this group was removed by a low-speed hand piece, then by a manual excavator until hearing sound of dentine scratching. Then the level of the surface was adjusted to the level of the cavity bottom by a diamond fissure bur applied to the edges of the cavity. Fourth group (control group): consisting of 10 newly extracted permanent molars free from caries. The occlusal enamel was cut by a diamond disc down to 0.5 mm below the dentino-enamel junction to obtain a smooth dentinal surface. The surface was smoothened by gradual glass discs under water cooling to obtain a very smooth dentine surface.

Preparation of Samples for Shear Bond Strength Examination

The teeth were placed in an acrylic model with the dentin surface remains exposed. Phosphoric acid was applied in a concentration of 37.5% for 15 seconds. After that the sample was washed with water for 5 seconds. Phosphoric acid was applied to all samples except second group in which we moved to the next stage directly without etching. A split acrylic model with 2 cm long and 2 cm wide and containing a cylinder hollow with a diameter of 2 mm and a height of 2 mm that serves as a matrix (acrylic matrix) to apply the restorative materials over the dentin (Fig 1). This matrix was fixed on the exposed dentin surface by adhesive wax and a celluloid strip was inserted inside (Fig 2). The bonding was done, and a gentle air was applied, and it was cured for 10 seconds. Then (Tetric® N-Ceram) composite was hold in the matrix at once and cured for 30 seconds by light-curing device (Fig 3). After that the acrylic model and celluloid strip were removed, and thus the four groups were ready for thermal cycles (Fig 4).

Study of Bond Strength

Application of Thermal Cycles

All samples were kept in physiological serum in a special incubator at a temperature of 37 ° C for 7 days. During this time, they were subjected to 500 thermal cycles ranging from 5-55 ° C according to (ISO TR 1145standard 1994) [14]. The duration of each cycle was 15 seconds for each degree, and the transfer time from one degree to another was approximately 8 seconds. We conducted the thermal cycles in the laboratory of the Faculty of Dentistry, using Memmert thermal cycle device. After finishing the thermal cycles, the shear tests were conducted.

Shear Bond Strength Tests

Shear strength were tested by INSTRON(1195) mechanical device, located in the Faculty of Mechanical Engineering, Damascus University. The samples to be measured were placed within a metal mold in the middle of the working table of the device. The head of the blade was applied at the bonding surface (between dentin on one hand and the restored material on the other hand) (Fig 5). The forces were applied at a speed of 5 mm/min until the sound of failure occurred and the results were read from the device panel that recorded the highest value reached by the sample at the moment of the failure and these results were recorded in the research chart.

Examination of the Bonding Surface under a Scanning Electron Microscope

After applying the shear strength tests by INSTRON device, the bonding surface was examined under a scanning electron microscope (Tescan Vega II XMU Variable Pressure) located in the Atomic Energy Authority with a magnification ranging between (300-3000) times to find out the type of failure occurring in the bonding surface. The following criteria were set by Burrow et.al 2003 [15] to assess the failure:

1- Failure at the level of bonding with dentin surface: 100% of the bonding surface failure between dentin and bonding material (we note the dentin tubes are opened) (Fig 6).
2- Failure at the level of bonding with restoration material: 100% of the bonding surface failure between bonding and resin composite (We note resin tags within the dentinal tubes) (Fig 7).
3- Failure at the level of the restorative material (composite resin): 100% of the failure is in the composite material (Fig 8).
4- Mixed failures: if the failure was compound between failure of bonding on the surface of dentin and failure at the level of the bonding or restorative material (Fig 9, 10).

STATISTICAL STUDY

The results were recorded and statistically processed using a program SPSS version 13.0 based on the analysis of variance ANOVA, and the binary comparisons by Bonferroni method. The significance of differences in the frequency of types of failure that occurred between the four groups was studied by means of chi-square test with the value of the significance level ($p = 0.05$).

RESULTS

Description of research sample: The research sample consists of 40 permanent human molars, which were divided into two parts. The first part contains 30 teeth, which in turn were divided into three equal groups according to the excavation method and the way of restoration material bonding. The second part (fourth group) is the control group of 10 teeth with intact dentine surfaces. After preparing the previous samples, shear strength tests were conducted to evaluate the effect of excavation technique on the bonding strength between dentin and composite resin. The surfaces of failure after shear strength were exam by scanning electron microscope to recognize the failure place. The results were as follow:

1- **Results of First Group**

The average shear bond strength for dentin samples that were etched and excavated by Carisolv then applying composite resin was (7.19 MPa) as shown in (Table No 2). The frequency of bonding failure was distributed as follows: 10% failure at the level of bonding with dentin surface, 50% failure at the level of bonding with restorative material, 20% failure at the level of restorative material, 20% mixed failure as shown in (Diagram No 3).

2- **Results of Second Group**

The average shear bond strength for dentin samples that were excavated by Carisolv without etching was (2.94 MPa). The frequency of bonding failure was distributed as follows: 60% failure at the level of bonding with dentin surface, 20% failure at the level of bonding with restorative material, 20% failure at the level of restorative material.

3- **Results of Third Group**

The average shear bond strength for dentin samples that were excavated in the traditional method was (5.22 MPa). The frequency of bonding failure was distributed as follows: 10% failure at the level of bonding with dentin surface, 50% failure at the level of bonding with restorative material, 20% failure at the level of restorative material, 20% mixed failure.

4- **Results of Fourth Group (Control Sample)**

The average shear bond strength of intact dentin samples with composite resin was (11.72 MPa) The frequency of bonding failure was distributed as follows: 30% failure at the level of bonding with dentin surface, 30% failure at the level of bonding with restorative material, 40% failure at the level of restoration material.

The Differences between the Types of Failure were Studied According to the Shear

Bond stress Results of the statistical study to compare between groups

A one-sided analysis of variance test ANOVA (Table No 2) demonstrate the presence of at least one of the average shear bond strengths is fundamentally different from other in the four studied groups ($p < 0.05$). To find out which of the averages differs from the other, the binary comparison was made according to Bonferroni method, (Table No 3). We found that at 95% confidence level, the shear bond strength in the healthy dentin group (the control group) was greater than the Carisolv group and the mechanical group. No statistically significant binary differences in the average shear bond strength between Carisolv group and Carisolv group without etching and mechanical excavation group, as well as there are no statistically significant binary differences in mean shear bond strength between the Carisolv group with etching and intact dentin group (Control group) in the research sample.

For the type of failure resulting from shear bond strength tests and its relationship to the method of excavation, preparation of dentin surface and the type of dentin surface either it is healthy dentin or residual dentin after excavation of caries. A chi-square test (Table No 4) Revealed no statistically significant differences ($p>0.05$) in the frequency of failure among the four groups studied in the research sample.

But to find out the possibility of a failure model more related to one of the used excavation methods, the standard residuals were calculated in (Table No 5). It was noted that there was one influential cell, which is the second cell, where the value of the standard residuals reached 3.1, which is greater than 2, This means that failure at the level of dentin tends to be associated with a Carisolv group without etching. To study the significance of differences in the average of shear bond strength between the groups of the type of failure, we conducted a one-sided analysis of variance ANOVA, (Table No 6). It was found that one of the average shear bond strengths is fundamentally different from another average in the corresponding failure type groups. To find out which of the averages differs from the other, a binary comparison was made according to the Bonferroni method, (Table No 7), The shear bond strength values in the failure at the level of restoration material were greater than the other failure groups (failure at the level of dentin, failure at the level of bonding and mixed failure). As for the rest of binary comparisons, we find that at 95% confidence level there are no statistically significant binary differences in the average shear bond strength between failure at dentin level, failure at the level of bonding and mixed failure in the research sample.

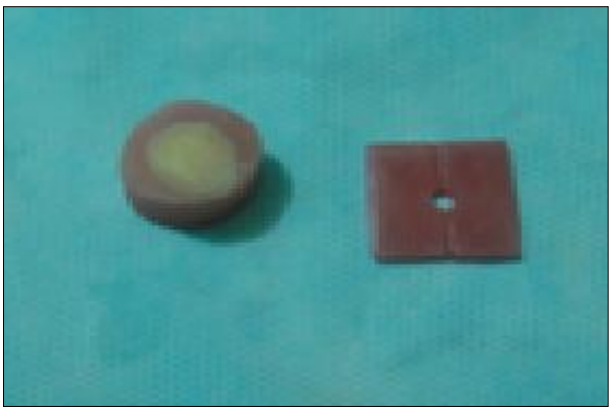


Figure 1: Acrylic Mode and the Sample

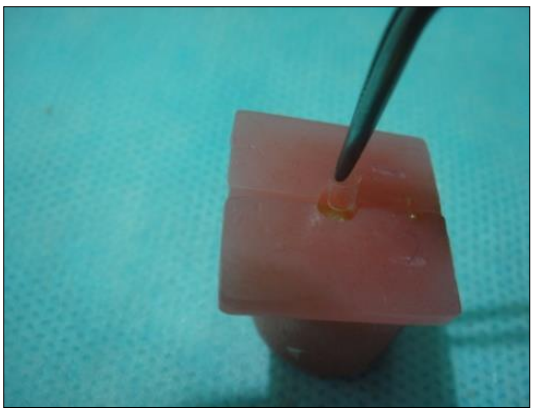


Figure 2: Applying Celluloid Strips

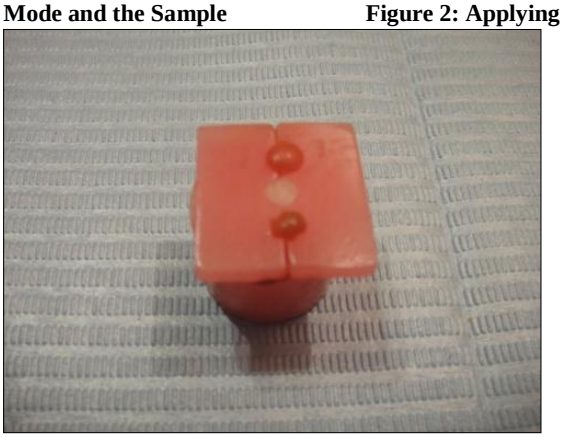


Figure 3: Applying Composite Inside the Acrylic Mode

Figure 4: The Sample after Applying the Composite

Figure 5: The Sample on the Instron

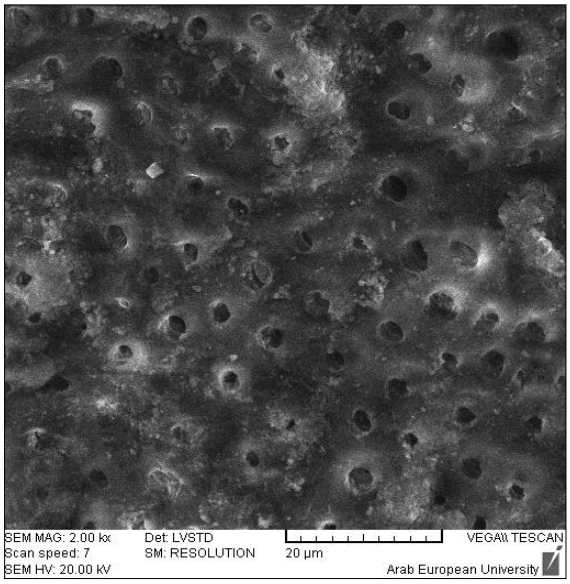


Figure 6: Failure at the Level of Bonding with Dentin Surface

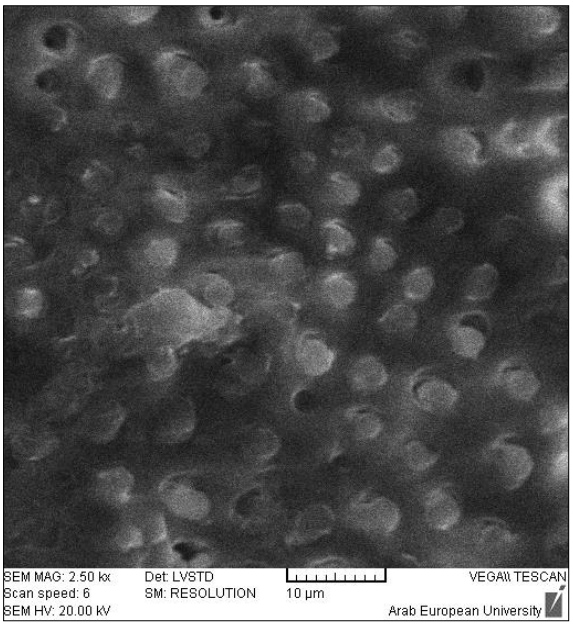


Figure 7: Failure at the Level of Bonding with Reatoration Material

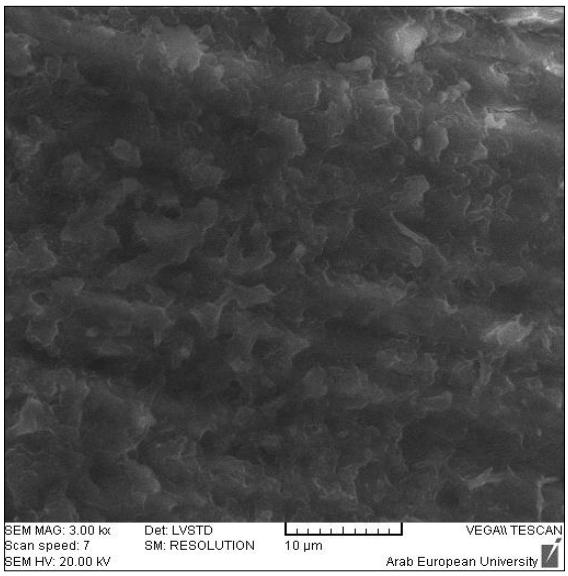
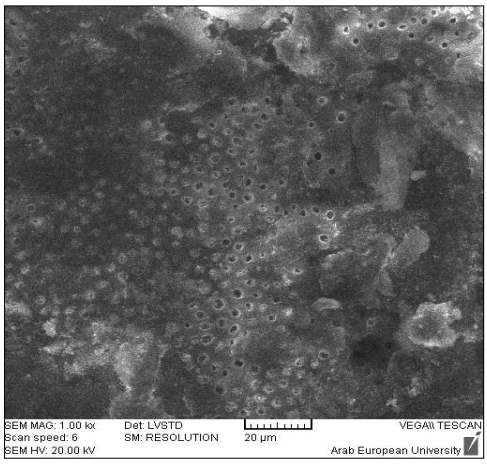


Figure 8: Failure at the Level of Restoration Material



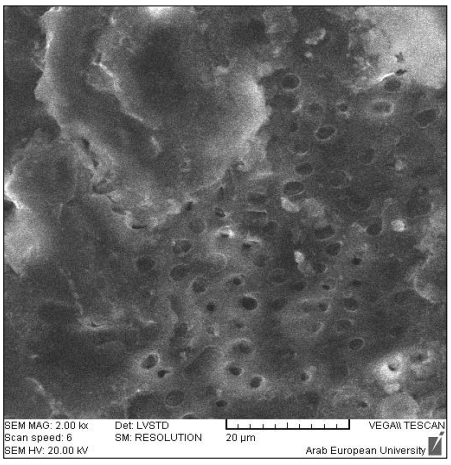
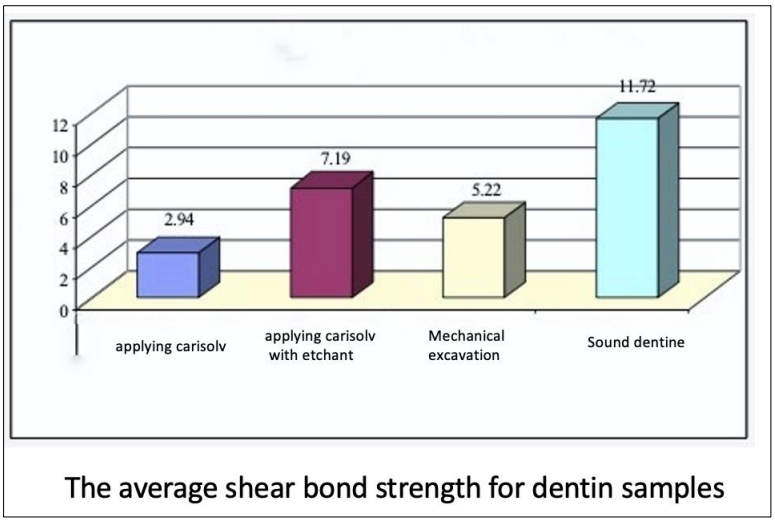


Figure 9: Mixed Failure Figure 10: Mixed Failur



The average shear bond strength for dentin samples

Diagram: 1

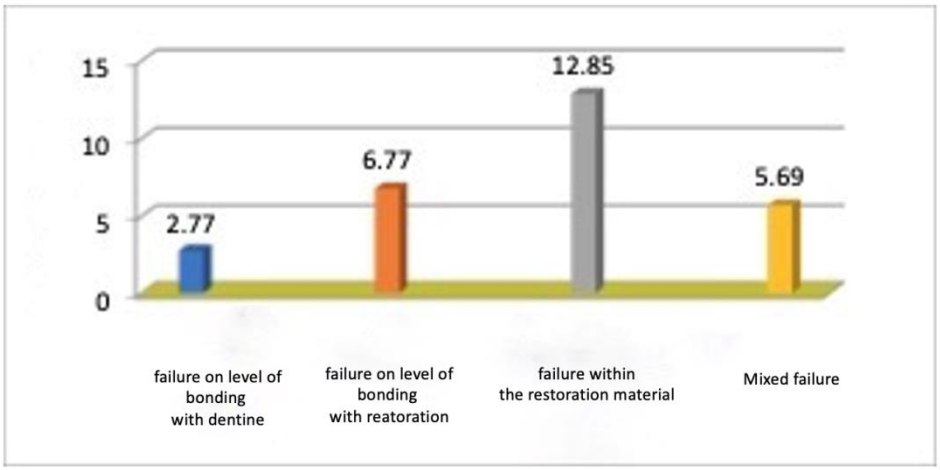


Diagram (2): The average shear bond strength for dentin samples related to the kind of failure

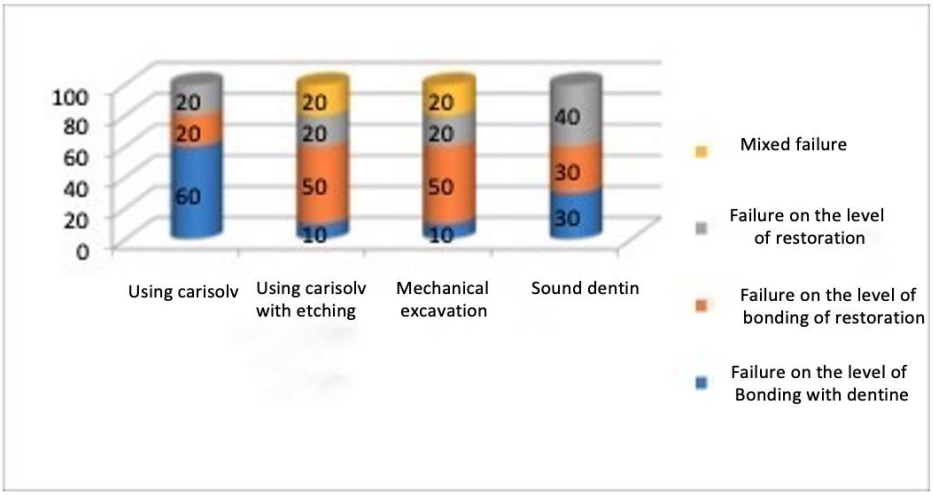


Diagram (3): percentage for the kinds of adhesive failure of research sample according to study groups

Table 1: Shows Arithmetic Mean, Standard Deviation, Standard Error, and Minimum and Highest Shear Bond Strength in MP According To the Studied Sample

Studied variable	Study groups	Teeth numbers	Arithmetic mean	Standard deviation	Standard error	Minimum value	Maximum value
Shear bond strength by MP	Excavation using Carisolv	10	2.94	1.09	0.35	1.25	5.00
	Excavation using carisolv with etchant	10	7.19	5.03	1.59	1.87	18.12
	Mechanical excavation	10	5.22	2.08	0.66	1.87	7.81
	Sound dentine control group	10	11.72	4.83	1.53	8.12	22.81

Table 2: Result of ANOVA Test to Know the Significance of Differences between Averages of Shear Bond Strength for the Four Groups

Studied variable		Sum of suares	Degrees of freedom	Contrast stimate	F	Significant Level value	Significance of differences
Shear bond strength in MP	Between groups	417.38	3	139.13	10.289	0.000	There is deferences
	Inside groups	486.76	36	13.52			
	total	**904.14**	**39**				

Table 3: Shows the Result of Binary Comparison According to Bonferroni to Study the Binary Differences in Shear Bond Strength between the Four Study Groups

Studied variable	group (I)	group (J)	Deference between Two averages (I-J)	Standard error	Significant Level value	Significant of differences
Shear bond strength MP	Excavation by carisolv	Excavation with carisolv Then using etching	-4.25	1.64	0.084	no differences
		Mechanical excavation	-2.28	1.64	1.000	No differences
		Sound dentin	-8.78	1.64	0.000	There is differences
	Excavation by Carisolve then use etching	Mechanical excavation	1.97	1.64	1.000	No differences
		Sound dentine	-4.53	1.64	0.055	No differences
	Mechanical excavation	Sound dentin	-6.50	1.64	0.002	There is differences

Table 4: Shows the Result of Chi-Square Test to Study the Mean of Failure Kinds in Study Groups

Tow study variables= study group/ kinds of failur				
Teeth number	Chi-square	Freedom degree	Significant Level value	Significant Of differences
40	13.343	9	0.148	Not significant

Table 5: Standard Residuals was calculated to know the Kind of Failure which is More Related to the Kind of Excavation

The group		الفشل				Total
Deference between	**Deference between**	**Deference between**	**Deference between**	**Deference between**	**Deference between**	**Total**
	number	2	5	3	0	10
	Expected value	2.5	1.8	4.0	1.8	10
	standard residuals	-.4	3.1	-.7	-1.7	
Excavation with carisolv the etching	number	2	1	5	2	10
	Expected value	2.5	1.8	4.0	1.8	10.0
	standard residuals	-.4	-.7	.7	.2	
Mechanical excavation	number	2	1	5	2	10
	Expected value	2.5	1.8	4.0	1.8	10.0
	standard residuals	-.4	-.7	.7	.2	

Sound dentin	number	4	0	3	3	10
	Expected value	2.5	1.8	4.0	1.8	10.0
	standard residuals	1.3	-1.7	-.7	1.2	
total	number	10	7	16	7	40
	Expected value	10.0	7.0	16.0	7.0	40.0

Table 6: Show the Result of ANOVA to Study the Differences in Shear Bond Strengths in MP between Groups of Kinds of Failure

Studied variable		Sum of squares	Degrees of freedom	Contrast estimate	F	Significance Level value	Significance of differences
Shear bond strength in MP	Between groups	398.67	3	132.89	9.464	0.000	There is differences
	Inside groups	505.47	36	14.04			
	Total	**904.14**	**39**				

Table 7: The Result of Binary Comparison According to Bonferroni to Study the Significance of Differences in Shear Bond Strength between Study Groups

Studied variable	Kind of failure (I)	Kind of failure (J)	Differences Between tow averages (I-J)	Standard error	Significant Level value	Significance of differences
Shear Bond Strength MP	Failure at the level of bonding with dentin surface	Failure at the level of bonding with dentin surface	-4.00	1.64	0.120	There is diffferences
		Failure at the level of bonding with restoration material	-10.08	1.94	0.000	No differences
		Mixed failure	-2.91	1.78	0.659	No differences
	Failure at the level of bonding with restoration material	Failure at the level of bonding with restoration material	-6.08	1.72	0.007	There is differences
		Mixed failure	1.08	1.53	1.000	No differences
	Failure at the level of bonding with restoration material	Mixed failure	7.17	1.85	0.003	There is differences

DISCUSSION

This study showed the superiority of composite bonding strengths with healthy dentinal tissues over bonding to remaining dentinal tissues after excavation of caries by the traditional method using slow-speed burs and hand excavators.

The bonding in smear-layer removal systems (total etch system used in this study) is based on the dual action of micro-mechanical tags of resin molecules in exposed collagen network fibers as well as the resin wedges extending within the open dentinal tubules [16]. It was noticed that the extension of resin tags and resin wedges in dentin layer after removing carious dentin is fewer than that observed in healthy dentin [17], due to the fact that the mass of remaining collagen fibers is immature and the severe lack of mineral crystals apatite in dentin remaining after caries removal that no longer fit into the voids Inside and outside the network of collagen and intracanal dentin [16]. Despite of severely lack of apatite crystals, a lot of remaining dentinal tubules after carious excavation was filled with mineral residues(B-octocalcium phosphate) caused by bacteria, which is not well bond as in natural dentin and is more resistant to acid [18,19]. Bonding was strengthened by the presence of chemical bonds between the carboxylate and phosphate radicals of methacrylate with dentin minerals, but the smaller number and larger crystals in the dentin affected by caries progress provide a less surface for adhesion [20]. Resulting is poor bonding. It was noticed that etching acid diffuses more easily in reaming dentin after caries removal due to its high porosity, which leads to a decrease in infiltration of monomer resins into the dentin and consequently a decrease in bonding strength [21].

The results of the current study agreed with Yoshiyama *et al*, [16], Burrow *et al*, [18] & Nakajama *et al*, [22], where they found that the bonding with the remaining dentin after excavation lower than the bonding with healthy dentin.

The results of this study differed with Sonoda *et al*, [2] where they found no difference in the bonding strength with healthy dentin or residual dentin after traditional caries excavation, due to the way of intact dentin surface preparation. Most studies make the surface of dentine polish and smooth but in Sonoda

study the surface of dentin was prepared by bur without polishing, which gives less bonding forces and this was confirmed by Ogata [23].

There were no statistically significant differences in the bonding strength of composite resin restorations with the remaining dentinal tissues after excavation by Carisolv or by the traditional method with low-speed burs and manual excavator or by Carisolv without itching. Although carisolv excavation with itching gave a higher non-statistically significant bonding strength, in addition to the absence of statistically significant differences with the healthy dentin. Wennerberg *et al,* [24] reported that the shape of the dentin tissue remaining after caries removal affects the bonding strength. A scanning electron microscope study to the remaining dentin surface after chemo-mechanical excavation showed an opening dentinal tubules in addition to a very small remnant of the smear layer covering the surface of the treated dentin [25, 26] and increase in surface roughness [27]. This may explain higher bonding strength in chemo-Mechanical method compared to the traditional mechanical excavation method. Koibuchi *et al,* [28] found that itched treated dentin surface in chemo-mechanical method has a higher surface energy compared to the itched treated dentin by traditional method. They explained that due to the presence of Naocl in the Carisolv with a pH=11, where sodium hypochlorite is used to obtain a bond between dentin minerals and bonding materials. Also, it affects the collagen fibers and thus enhance the integration of monomers within the demineralized dentin [29].

The current study agreed with Cehreli *et al*, [30] & Heng *et al*, [31], which found an increase in the bonding strength after cariesolv compared with traditional carious excavation without statistical significant differences between them.

The current study also differed with El Shehabyin [32]. Where she found that the bonding strength with the remaining dentin after mechanical excavation are higher than the bonding strength after chemo-mecanical method. This difference is due to the difference in the bonding system where the researcher used the self-itching bonding system in which high pH of Carisolv, equal to 11, affects the activity of the acid in the bonding system, which leads to a decrease in bonding strength. As for the relation of the failure type to the shear bond strength, the statistical study showed that the shear strength values in the failure group at the level of the restoration material were greater compared to other failure groups. This type of failure was the most visible in the healthy dentin group, followed by mechanical and chemo-mecanical group while it did not appear in the Carisolv group without a statistical difference in the frequency of this type of failure. The failure at the level of dentin is more associated with Carisolv group without itching due to the fact that the surface of the dentin remaining after carious removal has less retention of the bonding material than the surface of dentin itched with phosphoric acid, which was used with in other groups.

The results of our study agreed with Khattab and Omar study [33]. The failure at the level of the restorative material was compatible with the highest bonding strength of composite resin restorations with completely itched dentin surface, due to the microscopic mechanical retention of bonding in open dentinal tube orifices, creating high bonding forces.

CONCLUSIONS

Within the limits of this laboratory study, we conclude the following:

- There is difference in the bonding strength between healthy dentin and remaining dentin after caries excavation. We suggest to conduct any future study of the bonding strength on the remaining dentin after caries excavation as it is closer to the clinical case.
- Since the bonding strength of Carisolv group with itching did not show any statistical differences with the rest of the other groups, but it also did not show statistically significant differences with the healthy dentin group, which outperformed all the remaining groups, and therefore we recommend it ching the dentin surface after using carisolv material.

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