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THE SIGNIFICANCE OF THE POSITIONAL RELATIONSHIP BETWEEN THE SURFACE OF THE DENTIN SUBSTRATE AND THE BONDING AGENT ON THE BOND STRENGTH TO DENTIN

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ABSTRACT

60 non carious, extracted human teeth were used in this study, for evaluating the shear bond strength of composite resin to dentin using the all in one adhesive system (prompt L-Pop). All teeth were divided into four groups to represent four different surface substrate inclinations. Analysis of variance (ANOVA) test was performed to assess the presence of significant differences in mean shear bond strengths at different surface substrate inclinations (whether the different inclinations of surface substrate produced significant effect on the mean shear bond strengths). Student-Newman-Keuls test and the least significant difference procedure were used for pair wise comparison between means to find out which means were significantly different. The above tests were separately performed for the groups of specimens that were immediately investigated; those that were stored for one week and those that were stored for one month. ANOVA test, also, was performed to study the effect of time on the shear bond strengths of all groups of specimens at each substrate inclination through all time periods.

Results: Within each time period, the results revealed that different inclinations of surface substrate produced statistically significant changes in the mean shear bond strengths, ($P < 0.001$). The mean shear bond strengths of the group of specimens to which the application of the adhesive system was performed with the occlusal plane of the lower jaw of the phantom head parallel to the floor (group A_4), were significantly higher than the other 3 groups ($P < 0.05$). Group A_2 (the group of specimens to which the application of the adhesive system was performed with the occlusal plane of the upper jaw of the phantom head making a forty five degree angle with the floor), came next. The ranking of groups according to the mean shear bond strengths was : $A_4 > A_2 > A_1 > A_3$. Regarding the effect of storage time (one week and one month) on the mean shear bond strengths of the adhesive system used, it was found that there were no significant differences among the shear bond strengths of any of the tested groups, at any substrate inclination, through all time periods (T_0, T_1, T_2) ($P > 0.05$).

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INTRODUCTION

Bond strength measurement is one of the most common methods for evaluating the adhesive properties of restorative materials.[1] Today's patient pays more attention to cosmetics than ever before, and advances in dental adhesive technology have allowed the dentist to improve facial esthetics in a relatively simple way.[2]

The production of a perfect seal on the material tooth interface is one of the goals of restorative dentistry, in order to prevent the entrance of microorganisms and other contaminants into the environment, as well as to reproduce the lost peripheral seal of dentin.[3]

The strength and durability of adhesive bonds depend on several factors, among which are; the compositional and structural aspects of enamel and dentin, the intended method of dealing with the smear layer, wetting of the adhesive, polymerization shrinkage, transmission of functional stresses across the bonding interface, clinical variables and the types of adhesive and composite restorative material used.[1, 4-10]

Regarding, dentinal regions and the microstructural features of the dentinal substrate, the orientation of the dentinal tubules appears to be an important variable determining bond strength to dentin. This may be one of the reasons that the bond strengths of the resin are not uniform inside a cavity.[11]

Schupbach et al. [12], compared the morphology of the resin-dentin interface in areas where the dentinal tubules run perpendicular, or at an angle to the cavity surface with that of areas where they run parallel to it. They reported that the orientation of the dentinal tubules had a profound effect on the formation of the hybrid layer.

Wu et al. [13], reported that the contact surface area of the material may depend on the tubule orientation in the cavity wall to which the material is applied, and that the difference in contact surface may affect the seal provided by the filling material.

Phrukkanon et al.[14], compared the microtensile bond strengths of two adhesives to dentin as a function of tubule orientation on bond strength with either product and no significance was reported. However; Ogata.[15], claimed that the sample size used by Phrukkanon et al, was not enough, and he added that the sample size that he and his co-workers used was more than twice that used by Phrukkanon and his co-workers. Also Ogata stated that, the machining Phraukkanon and his co-workers used to make their specimens into cylindrical hour-glass shapes, might have created micro-cracks in the specimens. In their study, Ogata et al examined the effect of multiple applications of self-etching primer on regional tensile bond strength (μTBS) to artificial wedge – shaped cavities (i.e. occlusal vs gingival wall). They reported that the tensile bond strength to the gingival wall was significantly lower than to the occlusal wall (P0.05<). They attributed that to the fact that the arientation of the dentinal tubules with the occlusal wall was, generally, parallel to the prepared surface, while those of the gingival wall were perpendicular to the interface. Thus, there were more tubules connected to the cut surface at the gingival site than were seen at the occlusal site.

Ogata et al.[11], investigated the influence of the direction of dentinal tubules on resin-dentin tensile bond strength(μTBS). The results of that study were in accordance with those obtained from a similar previous study[15], by the same researchers: the tensile bond strength of the groups with tubules parallel to the bonded surface was higher than that of the tubules cut perpendicularly when the bond strength was measured on flat coronal dentin surfaces.

Ozer et al.[16], studied the shear bond strengths of composite resins to buccal dentin surfaces in comparison to those to the occluso-cervical dentin floors. It was found that, in most specimens, the bond strengths to buccal dentin surfaces were sig-

nificantly stronger that those to the occluso-cervical dentin floors.

Also several studies have reported that bond strength deteriorates over time. One of the factors that seemed to be involved with such deterioration, was the increase in porosity within the hybrid layer that would form nanoleakage pathways that were thought to permit fluid penetration within the hybrid layer.

Therefore, in light of all what was mentioned in this introduction, it might be relevant to study variations in the methodology regarding the use and application of adhesive systems.

So far, to the extent of the author's knowledge, no experimental studies have been conducted to assess any possible relationship between the direction of application of a particular adhesive and the resultant bond strength to dentin, which is the subject of this study and which can be come one of the most interesting issues under investigation.

METHODS AND MATERIALS

The materials used in this study are shown in table 1.

Specimens' preparation

60 non carious, extracted human first molars were selected. The teeth belonged to male patients of the age group (35 – 40) years old. Teeth were extracted due to periodontal reasons. All teeth, were stored in deionized water. Before usage, the teeth were cleaned and scrubbed then rinsed thoroughly.

Grouping of simples

All teeth were divided into four groups to represent four different surface substrate inclinations, and the influence of inclination of surface substrate on the shear bond strength was assessed as follows:

The shear bond strength of composite resin to dentin (60 teeth) after:

- Immediate investigation. (20 samples)
- After incubation for one week. (20 samples)
- After incubation for one month. (20 samples)

N.B incubation was achieved by placing the samples in a 100% humid environment.

* **The four different surface substrate inclinations: (A_1, A_2, A_3, A_4)**

All teeth were divided into four groups (A_1, A_2, A_3, A_4) The teeth belonging to each of the four groups were mounted on a cast by the aid of a compound impression material. Casts of the first three groups (A_1, A_2, A_3) were mounted on the upper jaws of three phantom heads, respectively, while the cast of the fourth group (A_4) was mounted on the lower jaw of a fourth phantom head.

The four different surface substrate inclinations (A_1, A_2, A_3, A_4) are shown in table 2.

** For the determination of the occlusal plane angulation to the floor, a ruler, a protractor and a right-angled triangle were used; e.g.: determination of a forty five degree angle, the long axis of the phantom head was set perpendicular to the

TABLE (1) Materials, Components, Batch numbers and manufactures:

Material	Components	Batch numbers	Manufacturer
Prompt™ L-Pop™ All-in-One Adhesive	L-Pop mixing / dispensing system containing liquid pre-dosed for one patient	440929	3M ESPE Dental Products St Paul, MN, USA
3M Filtek™ Z 250 Microhybrid composite	In organic filler: Zirconia/Silica (60% by volume) Matrix: BIS-GMA, UDMA and BIS-EMA resins	N361548	3M ESPE Dental Products St Paul, MN, USA

floor, The ruler was placed perpendicular to the long axis of the phantom head, to represent the horizontal axis (x). The right- angled triangle was placed along side the ruler to represent the vertical axis (y). Then, with both conjugate axes perpendicular, and a vertex (o) it was easy to determine a forty five degree angle to the floor *(represented by the x-axis)*, using the protractor.

TABLE (2) Factors to be investigated.

B	One-step technique adhesive system
A_1	The group of molars to which the application of the adhesive was performed with the occlusal plane of the upper jaw of the phantom head perpendicular to the floor.
A_2	The group of molars to which the application of the adhesive was performed with the occlusal plane of the upper jaw of the phantom head making a forty five degree angle to the floor.
A_3	The group of molars to which the application of the adhesive was performed with the occlusal plane of the upper jaw of the phantom head parallel to the floor.
A_4	The group of molars to which the application of the adhesive was performed with the occlusal plane of the lower jaw of the phantom head parallel to the floor.
T_0	Immediate usage.
T_1	After one week storage.
T_2	After one month storage.

TABLE (3) Interactions among variables.

n=5		T_0	T_1	T_2
B	A_1	BA_1T_0	BA_1T_1	BA_1T_2
	A_2	BA_2T_0	BA_2T_1	BA_2T_2
	A_3	BA_3T_0	BA_3T_1	BA_3T_2
	A_4	BA_4T_0	BA_4T_1	BA_4T_2

Total n = 60

Bonding Procedure

For each molar the occlusal surface was ground perpendicular to the long axis of the tooth, to expose a flat surface of dentin. Grinding was performed to the level of the DEJ. The dentin surface was then rinsed for 20 seconds with an air/water spray and gently air dried for 5 seconds. Afterwards the flat dentin surface was polished with # 600 silicon carbide paper under running water. The adhesive was then applied. The application of the adhesive system to the samples was according to the manufacturer's directions.

Direction of application

The direction of application of the adhesive system to samples belonging to each group is shown in table 2.

In addition it should be emphasized that for groups (A_1, A_2, A_3) the direction of application of the adhesive systems was against the general direction of the earth's gravity: (with the materials overcoming the pull of gravity), while for group (A_4) the direction of application of the adhesive systems was aligned in the general direction of the earth's gravity.

The resin composite application:

Composite resin was applied according to the manufacturer's instructions. The insertion of composite was achieved by the aid of polytetrafluoroethlyene molds each measuring 3 x 2 mm. Composite was then lightcured.

Specimens that were not to be immediately investigated, were incubated for one week and one month in a 100% humid environment.

Shear bond strength testing:

The samples were subjected to shear stress using a computer controlled materials testing machine (Model LRX-plus; Lloyd Instruments Ltd., Fareham, UK) with a loadcell of 5 kN and data were

recorded using computer software (Nexygen-4.1; Lloyd Instruments). *The bonded ring-dentin surface area was parallel to the shearing rod of the universal testing machine*.

Statistical analyses

Statistical analyses were performed using IBM â SPSS â Statistics Version 20 for windows. Statistical significance was achieved when the P-values ware £ 0.05.

Analysis of variance (ANOVA) test was performed to assess the presence of significant differences in mean shear bond strengths at different surface substrate inclinations (whether the different inclinations of surface substrate produced significant effect on the mean shear bond strengths).

Student-Newman-Keuls test and the least significant difference procedure were used for pairwise comparison between means to find out which means were significantly different.

The above tests were separately performed for the groups of specimens that were immediately investigated; those that were stored for one week and those that were stored for one month.

ANOVA test, also, was performed to study the effect of time on the shear bond strengths of all groups of specimens at each substrate inclination through all time periods.

RESULTS

Statistical analyses were performed using IBM â SPSS â Statistics Version 20 for windows. Statistical significance is achieved when the P-value is < 0.05.

1. Effect of Inclination of Surface Substrate on the Mean Shear Bond Strength of Prompt L-Pop:

(i) Before storage (at T0):

The means and standard deviations of the shear bond strengths of Prompt L-Pop adhesive at different surface substrate inclinations, before storage, are summarized in table (4) and figure (1).

TABLE (4) Mean shear bond strengths of Prompt L-Pop adhesive at different surface substrate inclinations, at T0.

Inclination (n = 5)	Shear Bond Strength (MPa) (Mean + Standard Deviation)
A1	16.2 + 1.3
A2	20.8 + 1.5
A3	13.7 + 1.2
A4	27.4 + 2

TABLE (5) ANOVA table for the shear bond strength of Prompt L-Pop adhesive at different substrate inclinations before storage.

Source of Variation	SS	DF	MS	F	P	Significance
Between Groups	543.14	3	181	75.4	<0.001	S
Within Groups	38.44	16	2.4			
Total	581.58	19				

SS: Sum of Squares, DF: Degrees of Freedom, MS: Mean Square, P < 0.001: Highly Significant, S.: Significant.

Analysis of variance (ANOVA) test was performed to assess the presence of significant differences in mean shear bond strengths at different surface substrate inclinations.

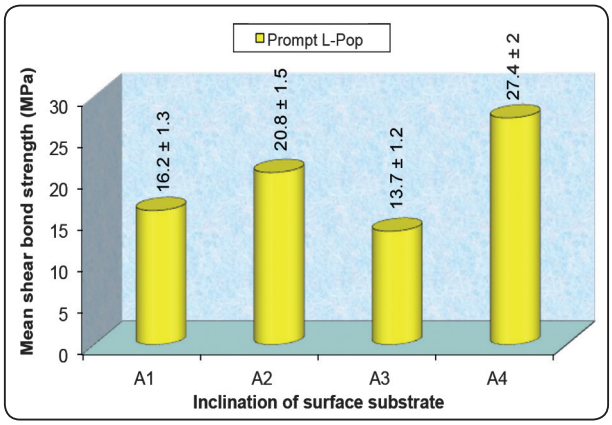

Fig. (1) Mean Shear Bond Strength to Dentin (MPa) at (T0)

The results were statistically significantly different ($P < 0.001$), as shown in table (5); meaning that different inclinations of surface substrate produced significant changes in the mean shear bond strengths.

Using Student-Newman-Keuls test and the least significant difference procedure (LSD), for pairwise comparison between means to find out which means were significantly different; it was found that the mean shear bond strengths of specimens belonging to groups (A4 & A3), (A4 & A2), (A4 & A1) and (A2 & A3); were statistically significantly different at $P < 0.05$; while specimens belonging to groups (A1 & A2) and (A1 & A3), demonstrated no statistical significance in regard to shear bond strengths, at $P < 0.05$.

The mean shear bond strengths of specimens belonging to group A4 were statistically significantly higher than those of specimens of the other 3 groups. Groups A1 & A3 demonstrated significantly lower mean shear bond strengths.

The ranking of groups according to the mean shear bond strengths was: A4 > A2 > A1 > A3

(ii) After One Week Storage (at T1)

The means and standard deviations of the shear bond strengths of Prompt L-Pop adhesive at different surface substrate inclinations, after one week storage, are summarized in table (6) and figure (2).

TABLE (6) Mean shear bond strengths of Prompt L-Pop adhesive at different surface substrate inclinations, at T1.

Inclination (n = 5)	Shear Bond Strength (MPa) (Mean + Standard Deviation)
A1	15.9 + 1.4
A2	20.5 + 1.8
A3	13.2 + 1.9
A4	26.9 + 2.2

TABLE (7) ANOVA table for the shear bond strength of Prompt L-Pop adhesive at different substrate inclinations after one week storage.

Source of Variation	SS	DF	MS	F	P	Significance
Between Groups	603.84	3	201.3	59.6	< 0.001	S
Within Groups	54	16	3.4			
Total	657.84	19				

SS: Sum of Squares, DF: Degrees of Freedom, MS: Mean Square, P < 0.001: Highly Significant, S.: Significant.

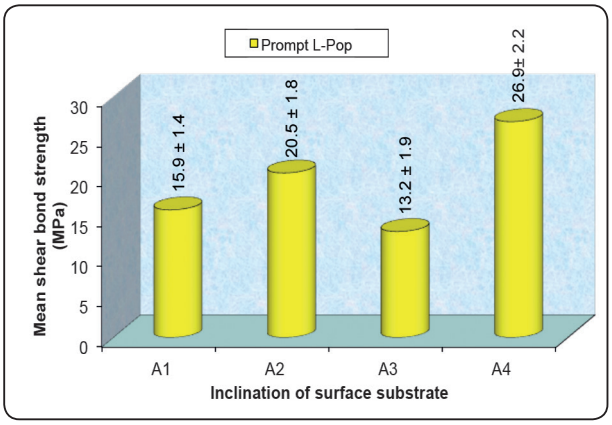

Fig.(2) Mean Shear Bond Strength to Dentin (MPa) at (T1)

Analysis of variance (ANOVA) test showed that the results were statistically significantly different ($P < 0.001$), as shown in table (7); meaning that different inclinations of surface substrate produced significant changes in the mean shear bond strengths.

Using Student-Newman-Keuls test and the least significant difference procedure for pairwise comparison between means it was found that the mean shear bond strengths of specimens belonging to groups (A4 & A1), (A4 & A2), (A4 & A3), (A2 & A1), (A2 & A3) and (A1 & A3), (i.e all groups); were statistically significantly different at $P < 0.05$.

The mean shear bond strengths of specimens belonging to group A4 were statistically significantly higher than those of specimens of the other 3 groups. Groups A1 & A3 demonstrated significantly lower mean shear bond strengths.

The ranking of groups according to the mean shear bond strengths was: A4 > A2 > A1 > A3.

(iii) After One Month Storage (at T2):

The means and standard deviations of the shear bond strengths of Prompt L-Pop adhesive at different surface substrate inclinations, after one month storage, are summarized in table (8) and figure (3).

TABLE (8) Mean shear bond strengths of Prompt L-Pop adhesive at different surface substrate inclinations, at T2.

Inclination (n = 5)	Shear Bond Strength (MPa) (Mean + Standard Deviation)
A1	14.8 + 2.6
A2	19.1 + 2.8
A3	12.5 + 2.2
A4	25.3 + 4

TABLE (9) ANOVA table for the shear bond strength of Prompt L-Pop adhesive at different substrate inclinations after one month storage.

Source of Variation	SS	DF	MS	F	P	Significance
Between Groups	474.84	3	158.3	17.7	< 0.001	S
Within Groups	142.8	16	8.9			
Total	617.64	19				

SS: Sum of Squares, DF: Degrees of Freedom, MS: Mean Square, P < 0.001: Highly Significant, S.: Significant.

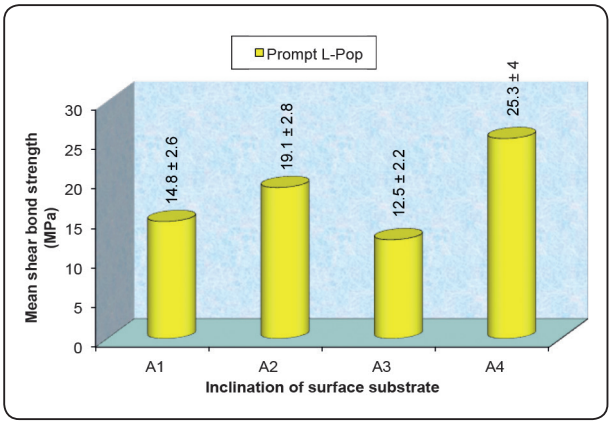

Fig. (3) Mean Shear Bond Strength to Dentin (MPa) at (T2)

Analysis of variance (ANOVA) test showed that the results were statistically significantly different (P < 0.001), as shown in table (9); meaning that different inclinations of surface substrate produced significant changes in the mean shear bond strengths.

Using Student-Newman-Keuls test and the least significant difference procedure (LSD), for pairwise comparison between means to find out which means were significantly different; it was found that the mean shear bond strengths of specimens belonging to groups (A4 & A3), (A4 & A2), (A4 & A1), (A1 & A2) and (A2 & A3); were statistically significantly different at P < 0.05; while specimens belonging to groups (A1 & A3), demonstrated no statistical significance in regard to shear bond strengths, at P < 0.05.

The mean shear bond strengths of specimens belonging to group A4 were statistically significantly higher than those of specimens of the other 3 groups. Groups A1 & A3 demonstrated significantly lower mean shear bond strengths.

The ranking of groups according to the mean shear bond strengths was: A4 > A2 > A1 > A3.

2. Effect of Time on the shear Bond Strength:

For the adhesive system used, analysis of variance (ANOVA) test was performed for each

surface substrate inclination (A1, A2, A3, A4), to compare the mean shear bond strengths of the adhesive system through all time periods (T0, T1, T2).

The results were not statistically significant ($P > 0.05$), meaning that there were no significant differences among the shear bond strengths of the adhesive system used at any surface substrate inclination, through all time periods. (Tables: 10, 11, 12, 13).

TABLE (10) ANOVA table for the mean shear bond strengths of Prompt L-Pop adhesive at inclination A1 through all time periods.

Source of Variation	SS	DF	MS	F	P	Significance
Between Groups	5.4	2	2.7	0.78	0.5	N.S.
Within Groups	41.5	12	3.46			
Total	46.9	14				

SS: Sum of Squares, DF: Degrees of Freedom, MS: Mean Square, N.S: Non Significant.

TABLE (11) ANOVA table for the mean shear bond strengths of Prompt L-Pop adhesive at inclination A2 through all time periods.

Source of Variation	SS	DF	MS	F	P	Significance
Between Groups	8.23	2	4.1	0.95	0.4	N.S.
Within Groups	51.98	12	4.3			
Total	60.21	14				

SS: Sum of Squares, DF: Degrees of Freedom, MS: Mean Square, N.S: Non Significant.

TABLE (12) ANOVA table for the mean shear bond strengths of Prompt L-Pop adhesive at inclination A3 through all time periods.

Source of Variation	SS	DF	MS	F	P	Significance
Between Groups	3.6	2	1.82	0.55	0.6	N.S.
Within Groups	39.9	12	3.33			
Total	43.5	14				

SS: Sum of Squares, DF: Degrees of Freedom, MS: Mean Square, N.S: Non Significant.

TABLE (13) ANOVA table for the mean shear bond strengths of Prompt L-Pop adhesive at inclination A4 through all time periods.

Source of Variation	SS	DF	MS	F	P	Significance
Between Groups	17.1	2	8.55	1	0.4	N.S.
Within Groups	101.8	12	8.5			
Total	118.9	14				

SS: Sum of Squares, DF: Degrees of Freedom, MS: Mean Square, N.S: Non Significant.

DISCUSSION

In this study, the effect of inclination of surface substrate on the shear bond strength of composite resin to dentin using the adhesive system prompt L-Pop was investigated. Prompt-L-Pop simultaneously demineralizes and penetrates into dentin, thus eliminating the problem of discrepancy between the depth of demineralization and the depth of monomer diffusion.[6]

Regarding the quality of the hybrid layer, the depth of demineralization and the depth of monomer diffusion have to be considered. A discrepancy,

between the depth of etch and the depth of resin penetration adversely affects the bond strength. Therefore, no discrepancy should exist between demineralization and infiltration. They should offer a twofold bonding mechanism based on micro-mechanical interlocking through hybridization to resist "acute" debonding stress, and an improved monomer-collagen interaction, which may be helpful to keep the bonds leakage-free in a long term perspective. [5, 6, 17, 18, 19]

Analysis of variance (ANOVA) test was performed to assess the presence of significant differences in mean shear bond strengths at different surface substrate inclinations.

For the adhesive system used, and within each time period, the results revealed that different inclinations of surface substrate produced statistically significant changes in the mean shear bond strengths. The significance was considered as high ($P < 0.001$).

The second step in investigation was to compare the means of shear bond strength of the groups of specimens representing, the four different inclinations (A1, A2, A3, A4). Student-Newman-keuls test and the least significant difference procedure (LSD), were used for so.

For the adhesive system used, and within each time period it was found that the mean shear bond strengths of specimens belonging to groups (A4 & A3), (A4 & A2), (A4 & A1), (A3 & A2) and (A2 & A1), were statistically significantly different at $P < 0.05$, while specimens belonging to groups (A1, A3), in most instances, demonstrated no statistically significant differences regarding the mean shear bond strengths, ($P < 0.05$).

The mean shear bond strengths of the group of specimens to which the application of the adhesive system was performed with the occlusal plane of the lower jaw of the phantom head parallel to the floor (group A4), were significantly higher than the shear bond strength's means of the other three groups ($P < 0.05$).

The ranking of groups according to the mean shear bond strengths was : A4 > A2 > A1 > A3.

Group A2 (the group of specimens to which the application of the adhesive system was performed with the occlusal plane of the upper jaw of the phantom head making a forty five degree angle with the floor), came next.

The fact that the inclination of the dentinal surface substrate appeared to have a highly significant effect on the shear bond strengths, would suggest that such a surface inclination probably would have affected the quality of the hybrid layer in terms of : degree and direction of infiltration of adhesive monomers with in the complex anatomy of the dentinal substrate (qualitative distribution); resistance to debonding; mechanical properties of the hybrid layer (elastic modulus, tensile strength and fracture toughness) and nanoleakage.

Recently, dentinal tubules anatomists has been suggested as a potential factor in adhesive bonding. Lateral side-branches of dentinal tubules were described as filled with polymerized adhesive resin. Also, the extension of the hybrid layer into the tubule wall area (Resin tag formation in the opened tubules, was described as circularly surrounded by a hybridized tubule-orifice wall), was thought to be favorable in hermetically sealing the puplo-dentinal complex against micro leakage. Moreover, it was found that that effect might be especially protective when the bond failed either at the bottom or top of the hybrid layer, which were considered the two weak links in the micromechaincal attachment. Afterwards the resin tags usually broke off at the hybrid layer surface keeping the dentinal tubules and hence the direct connection to the pulp sealed. In particular, the resin-tag necks at the top 5-10 μm of the tubule orifices were thought to contribute most to retention and sealing effectiveness. [20, 21, 18, 6, 11]

According to the Elastic bonding concept, the hybrid and adhesive layers may act as strain-absorbing layers in bonding systems. Dentin resin interface is comprised of several layers of materials with different mechanical properties. The elastic modulus of the successive layers across a resin-dentin bonding area is markedly influential. A gradient of elastic modulus exists from the rather stiff dentin over a more flexible hybrid layer and bonding agent layer to the stiffer resin composite. Single application bonding systems create a rather thick layer between the restoratives and tooth substrate. A thick layer of the adhesives of the single application bonding systems within sub-micron filler addition might assist in close adaptation of the restoration without gap formation. (5)

In this study, the effect of storage time (one week and one month) on the mean shear bond strengths of the adhesive system used was assessed.

Analysis of variance (ANOVA) test was performed for each surface substrate inclination (A1, A2, A3, A4), to compare the mean shear bond strengths of the adhesive system through all time periods (T0, T1, T2).

The results were not statistically significant ($P > 0.05$), meaning that there were no significant differences among the shear bond strengths of the adhesive system used at any surface substrate inclination, through all time periods.

The durability of bonds between the adhesive resins and dentin is of critical importance for the longevity of bonded restorations. Several reports evaluated the durability of dentin bonds in vitro. The reports showed that dentin bond strength decreased in water storage after several years. Degradation of bond strength might result from the plasticizing effect of water on resin and collagen (22), water sorption and / or hydrolysis adhesive resin, or hydrolysis of collagen fibrils at the base of the hybrid layer.

Although it was reported that in vivo bond strengths were relatively stable over several years, yet it was observed that porosity of the hybrid layer increased over time. (10, 17)

Concern remains regarding the long-term durability of resin bonds since degradation of the resin-dentin interface may result from hydrolytic attack of resin through ever enlarging nanoleakage pathways.

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