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Effects of mechanical and thermal load cycling on micro tensile bond strength of clearfil SE bond to superficial dentin

Ali Reza Daneshkazemi[1], Abdol Rahim Davari[1], Ebrahim Ataei[2], Fariba Dastjerdi[3], Ehsan Hajighasemi[4]

[1]Department of Operative Dentistry, Member of Social Determinants of Oral Health Research Center, [2]Department of Operative Dentistry, [3]Department of Pediatric Dentistry, School of Dentistry, Shahid Sadoughi University of Medical Sciences, [4]Private Practice, DDS, Yazd, Iran

ABSTRACT

Background: Certain studies have been conducted on the effects of mechanical and thermal load cycling on the microtensile bond strength (microTBS) of composites to dentin, but the results were different. The authors therefore decided to evaluate these effects on the bonding of Clearfil SE bond to superficial dentin.

Materials and Methods: Flat dentinal surface of 42 molar teeth were bonded to Filtek-Z250 resin composite by Clearfil SE bond. The teeth were randomly divided into 7 groups and exposed to different mechanical and thermal load cycling. Thermocycling was at 5-55°C and mechanical load cycling was created with a force of 125 N and 0.5 Hz. Then, the teeth were sectioned and shaped to hour glass form and subjected to microTBS testing at a speed of 0.5 mm/min. The results were statistically analyzed by computer with three-way analysis of variance and *T*-test at $P < 0.05$ significant. To evaluate the location and mode of failure, the specimens were observed under the stereomicroscope. Then, one of the specimens in each group was evaluated under Scanning Electron Microscopy (SEM) for mode of failure.

Results: All of the study groups had a significantly lower microTBS as compared to the control group ($P < 0.001$). There was no statistically significant difference between mechanical cycling with 50K (kilo = 1000) cycles, and 50K mechanical cycles plus 1K thermal cycles. Most of the fractures in the control group were of adhesive type and this type of fracture increased after exposure to mechanical and thermal load cycling.

Conclusion: Thermal and mechanical load cycling had significant negative effects on microTBS and the significant effects of mechanical load cycling started to be significant at 100K cycles.

Key Words: Adhesive, dentin, mechanical cycling, micro tensile bond strength, thermal cycling

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Address for correspondence:
Dr. Abdol Rahim Davari, Department of Operative Dentistry, School of Dentistry, Shahid Sadoughi University of Medical Sciences, Dahe Fajr BLVD, Yazd, Iran.
E-mail: rdavari2000@yahoo.com

INTRODUCTION

Light cure resin composites have been used for a long time in restorative dentistry to restore dental structure and correction of color and contour of the teeth. Studies on the adhesion of resin composites to teeth started with the studies on the adhesion to enamel and followed by to dentin.[1]

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To date, seven generations of dentin-bonding agents have been introduced. The sixth generation of bonding agents (self-etch) needs fewer stages and is easy to apply. This bonding agent has been made on the basis of simultaneous use of conditioner and primer on enamel and/or dentin with the help of non-washable acid monomers. Most of these bonding agents are composed of two phases. In the first stage, condi-primer and in the second stage, adhesive is applied on dentinal surface.[2] The bonding agent used in the present study belongs to this category of adhesive systems.

In order to evaluate the characteristics of bonding, most of the researchers have usually performed *in-vitro* studies and simulated thermal and mechanical load cycling to resemble the oral environment.[3]

Nowadays, microtensile bond strength (microTBS) is used to evaluate the bond strength of resin composites to dental hard tissues in the extra oral environment.

Nikaido evaluated the microTBS and mode of fractures of resin composite restorations after application of thermal and mechanical load cycling on 24 molar teeth. In first group, flat dentinal surfaces were made and following the use of an adhesive, the crowns of the teeth were built-up with resin composite. In second group, class I cavities were prepared and then restored using two types of adhesives and resin composite. The samples of both groups were divided into 4 subgroups and were exposed to mechanical load cycling for 0, 1K, 5K, and 10K cycles with 50 N (Newton) load, and thermocycling for 0, 125, 625, and 1250 cycles. The samples were then immersed in water for 1 week and exposed to microTBS test. The location of bond failure of resin composites in each sample was examined by SEM. The results showed that the mean microTBS in first group, was approximately 40 MPa, and so, mechanical and thermal load cycling did not affect the microTBS. In second group, the mean of microTBS in the control group was 21 MPa and decreased significantly in the other groups as the cycles of thermal and mechanical load were increased.[4]

Mitsui studied the effects of mechanical and thermal load cycling on the microTBS in total-etch and self-etch adhesive systems. Class II cavities were prepared in 168 bovine incisor teeth and restored with resin composites using self-etch and total-etch dentin bonding agents and composite. The teeth were then divided into 7 equal groups and various thermal and mechanical load cycling were applied. For the thermocycling, water baths at 5°C and 55°C with 60 s of dwell time were used and mechanical cycle was performed with 80 N at 2 cycles/s. Then, the samples were sectioned and trimmed to obtain a surface of 0.81-1 mm^2 and tested for microTBS. The results showed that total-etch adhesive had significantly higher microTBS than that of self-etch adhesive and the bond strength decreased as the rate of load cycling were increased, but at 100K load cycle, there was no significant difference in bond strengths compared to the control group.[5]

Xie evaluated the effect of thermocycling on microTBS of one- and two-step self-etching adhesives. Clearfil S3 Bond (S3) and Clearfil SE Bond (SE), were applied on cervical lesions in human premolars and restored by using Clearfil AP-X resin composite. Then the teeth were sectioned into 0.7 × 0.7 mm composite-dentin beams and aged with 0, 5K, or 10K thermocycles. The beams were subsequently subjected to microTBS testing at a crosshead speed of 1 mm/min and statistical analyses were computed. The results showed statistically significant effects on bonding effectiveness by adhesive system, thermocycling, or combinations of the adhesive system and thermocycling ($P < 0.05$). Regardless of the lesion type, the microTBS for S3 decreased significantly after 5K or 10K thermocycles, while the microTBS for SE showed a significant decrease only after 10K thermocycles. The results suggested that thermocycling had a significant negative effect on the bond strength of the two SEAs tested.[6]

Therefore, considering the results of the above-mentioned studies, the aim of present study was to evaluate the effects of mechanical and thermal load cycling on the microTBS of a self-etch dentin bonding agent (Clearfil SE Bond) to superficial dentin and also to observe the modes of fractures.

MATERIALS AND METHODS

Forty-two extracted sound maxillary molar teeth without caries and developmental defects were collected over a period of 1 month. The teeth were stored in normal saline in the room temperature.[7] Then debridement was done for removing the adjacent periodontal tissues. In order to carry out infection control, the teeth were disinfected in 0.5% choloramine-T solution for 24 h prior to study.[8] Then, a diamond burr (SS White/USA) in high-speed handpiece with water spray was used to remove the enamel and expose the underlying dentin and after 5 time tooth preparations, another burr was used. Before application of the dentin-bonding system, the dentin surfaces were polished by 320 grit silicone adhesive paper to create standard smear layer on each tooth surface,[9] and the teeth were then washed under tap water and the excess water was removed. After preparation of tooth surfaces, the adhesive system used in this study, Clearfil SE Bond (Kuraray Co, Osaka/Japan), was applied to dentin surfaces according to the manufacturer's instructions. The bonding primer of Clearfil SE Bond (Kuaray/Japan) was applied on the prepared dentinal surface by a microbrush according to the instructions of the manufacturing company and allowed to be remained for 20 s before spreading it with gentle air stream. The primer then was light cured using Astralis light cure unit (Vivadent/Lichtenstein) with an intensity of 500 mw/cm^2 for a period of 10 s. The intensity of light had been confirmed by a radiometer (Dentamerica/Taiwan).[7] Then, Filtek Z_{250} resin composite (3M, Dental Product, St Paul, MN/USA) was applied on the bonded area in two layers of 1.5 mm. Each layer was irradiated with Astralis curing light unit, separately, on four sides for a period of 40 s. The distance between the tip of the light source and resin composite was at the minimum distance and the head of the light cure unit was holding perpendicular to the surface of the composite restoration. This distance was maintained for all samples. The teeth were then randomly divided into 7 groups (G1-G7) of 6, and the study went on as follows:

Samples were mounted in self-curing acrylic resin (Flash Acrylic, Yates Motloid, Chicago, IL/USA) to a level 1 mm below the CEJ of every tooth and then, Mechanical load cycling of 0, 50K, 100K and 500K were applied on groups G1 through G7, respectively, and thermocycling were applied to G5 through G7 groups for 1K cycles.

For mechanical load cycling, the teeth were mounted in the mold of the Load cycling machine (Vafaii Corp./Iran). The distance between the force area of the machine and each tooth was adjusted and then the force was applied. During mechanical load cycling, the teeth were immersed in normal saline. It is worth mentioning that the magnitude of applied force on the teeth in the mechanical cycles was 125N with a frequency of 0.5 HZ.[3] Group G5 through G7 specimens were thermocycled between 5°C and 55°C with a holding and dwell time of 15 s and 60 s respectively.[5] The teeth were then placed in the mold filled with self-cure acrylic resin (Flash Acrylic, Yates Motloid, Chicago, IL/USA) in the appropriate position. A 0.3 mm diamond disk (Ham Co. Machines, Inc., Rochester/USA) was used to cut the teeth in mesiodistal direction and parallel to the horizontal plane of the teeth under running water to prepare 1-mm thick slabs of teeth. A total of 12 samples were made in each group. Using a diamond fissure burr (SS White/USA), the segmented samples were thinned at the bonding area to create an hour-glass shape with an interface area of 0.8-1 mm^2. Then, the samples were subjected to microTBS test by universal Testing machine (Bisco Corp./USA) at a cross head speed of 0.5 mm/min in order to create fracture and the applied force was recorded. The results were analyzed by analysis of variance (ANOVA) and *T*-test using $SPSS_{16}$ software program. In order to determine the mode of fracture, the samples were examined by a stereomicroscope (Zeiss-Stenc-SV11/ Germany) with ×20 magnification and one sample in each group was evaluated by SEM (Philips, XL20/ Netherlands).

RESULTS

Kolmogorov–Smirnov test confirmed the normal distribution of data in all groups (G1 = 0.972, G2 = 0.981, G3 = 0.974, G4 = 0.949, G5 = 0.802, G6 = 0.763, G7 = 0.876) Also, regarding the similarity in variance of the groups, based on Levene tests ($P = 0.66$), ANOVA was used to compare the groups.

The results showed that the highest mean value of microTBS was in G_1 (35.4 Mpa), while the lowest was in G_7 (12.71 Mpa) respectively. The difference between all groups was significant ($P < 0.001$) [Table 1]. The difference between the test and control groups according to Dunnett *post hoc* test was statistically significant ($P < 0.001$).

In the present study, with an increase in the cycles of the mechanical load, with and without thermocycling, microTBS values decreased significantly [Table 2]. Results of the two-way analysis of variance showed that both mechanical and thermal load cyclings had an effect on the microTBS and also, there was a reciprocal difference between the effects of mechanical and thermal load cycling [Table 3].

In the present study, all of the fracture sites were studied by a stereo microscope with a magnification of ×20. The mode of fracture evaluation showed that the maximum number of fractures was in the adhesive (64.28%) and the minimum was, mixed type (9.52%) respectively [Table 4].

Table 1: Mean value and standard deviation of microtensile bond strength in groups in Mpa scale

Group	Number	Mean	SD	*P*
G_1	12	35.4	2.30	<0.001
G_2	12	31.5	1.53	
G_3	12	28.05	1.66	
G_4	12	18.13	1.72	
G_5	12	30.74	1.70	
G_6	12	25.45	1.61	
G_7	12	12.71	1.66	

Moreover, the type of fractures in one of the samples of each group was studied by SEM.

DISCUSSION

The present study was conducted with the aim of studying the effects of mechanical and thermal load cycling on the microTBS of Clearfil SE bond to superficial dentin. Results determined that application of simultaneous mechanical and thermal cycling leads to a decrease in microTBS that is consistent with the studies by Bedran De Castro *et al.*,[10,11] Toledano *et al.*,[12] Mitsui *et al.*,[5] Abdalla *et al.*,[3] and Kasraei and Khamverdi.[8] The results of the study were not consistent with the results of Nikaido *et al.*[4] that showed after 50K mechanical load and 2K thermal cycles, there was no significant difference in the values of microTBS between the study and control groups. This may be explained by the fact that, the applied pressure on resin composite in their study might be eccentric. Also, resin composite could act as a shock absorber and distributed the force during mechanical loading. Other factors including type of tooth, adhesive agent, the time passed since extraction, environmental circumstances, and intensity and direction of applied force to the samples could play a role in the outcomes of that study.

In the present study, the effects of 1K thermal cycle became significant at mechanical cycles more than 50K, whereas in the study by Nakata *et al.*,[13] the effect of 1K thermal cycles was not significantly different from that of the control group.

Results of the most clinical studies are consistent with the most *in-vitro* studies, but due to some limitations, it is not possible to simulate the oral environment in a laboratory. Therefore, many studies have used methods like mechanical load cycling and thermo cycling in order to achieve conditions similar to oral environment.[14] In the present study, simultaneous application of mechanical and thermal load cycling were used to mimic chewing condition too. Thermo cycling is a common method to simulate oral environment in laboratory. On the basis of International Standard Organization (ISO) TB 11450 standard, 500 cycles must be carried out for thermo cycling.[15] Based on a review article, a thermo cycling of 1K is similar to approximately 1 year work in mouth environment and the 500 cycles proposed by ISO standard is very minimal in mimicking the long term.[15] But other studies have reported different thermo cycles to mimic the aging of dental materials. The researches placed teeth or restorations at a temperature comparable to the oral cavity and applied stress on bonding area.[16] This process helped us to understand stress generation in restoration due to aging of restoration and thermal changes.[17] Changes in thermocycles lead to speeding up the hydrolysis of unprotected collagen fibers by high-temperature

Table 2: Variance analysis of both sides without considering the control group

Thermal cycle/ Mechanical cycle	With thermocycling		Without thermocycling		*P*
	Mean	SD	Mean	SD	
50K	30.74	1.70	31.5	1.53	0.264
100K	25.45	1.61	28.05	1.66	<0.001
500K	12.71	1.66	18.13	1.72	<0.001
P	<0.001		<0.001		

Table 3: Variance analysis of both sides without considering the control group

Effect of mechanical and thermal factors	Degree of freedom	*F*	*P*
Effect of mechanical load cycling	2	576.77	<0.001
Effect of thermal load cycling	1	56.4	<0.001
Opposite effect of mechanical and thermal load cycling	2	12.09	<0.001

Table 4: Distribution of the mode of failure in samples

Type of fracture/Group	Adhesive		Cohesive		Mix	
	Distribution	Percentage	Distribution	Percentage	Distribution	Percentage
G_1	6	50	5	41.66	1	8.33
G_2	7	58.33	4	33.33	1	8.33
G_3	7	58.33	4	33.33	1	8.33
G_4	8	66.66	2	16.66	2	16.66
G_5	7	58.33	4	33.33	1	8.33
G_6	9	75	2	16.66	1	8.33
G_7	10	83.33	1	8.33	1	8.33
Total	54	64.28	22	26.19	8	9.52

water and removing the resin oligomers that are not properly polymerized.[18-20] Also, because of the higher rate of thermal expansion of restorative materials compared to dental structures, repetition of contraction and expansion leads to formation of a gap between tooth and restoration. Changes in the size of the gap can result in pathological fluid movement that causes microleakage and is most severe at the bonded area.[21,22] From a clinical point of view, these are the most vulnerable margins.[15]

Repetitive contractive/expansive effect of thermocycling results in formation of stresses similar to clinical conditions and when the ratio of C-factor gets high, more stress is created.[23] It is not clear whether the thermocycling has an effect on the bonding strength, although one meta-analysis study showed that thermocycling does not have a significant effect on the bond strength.[24]

Intra-oral restorations are continuously exposed to stresses from the opposite teeth for about 1 million mechanical strokes per year.[14] These strokes over a long period of time have their effects on bonds of the interface between restoration and tooth surface and result in weakening failure of the restoration.[4]

In the present study, the mechanical cycles applied to the teeth were 50K, 100K, and 500K, respectively. Different studies have reported different mechanical cycles. For example, Nikaido *et al*.[4] used 10K, 50K, and 100K. Bedren De Castro *et al*.[10,11] and Kasraei and Khamverdi[8] used 100K, Toledano *et al*.[12] and Mazzitelli *et al*.[25] used 5K and Abdolla *et al*.[3] applied 4K cycles in their studies. The present study is different from them regarding the variety in number of mechanical cycles.

Anderson[26] reported that, the appropriate load during chewing and swallowing is between 70 N and 150 N. Various studies applied different forces[3-5,10-12] for example, Nikaido *et al*.[4] and Bedran De castro *et al*.[10,11] applied 50, Tolendano *et al*.[12] 90, Mitsui *et al*.[5] 80, Abdalla *et al*.[3] 125 and Kasraei and Khamverdi[8] 60 N, but in the present study, 125 N force was loaded that is one of the highest forces exerted on the teeth.

In the last two decades, one of the most important topics in restorative dentistry has been determination of appropriate methods to bond resin composite to dental hard tissues. Appropriate bonding is in relation to chemical, physical, and mechanical properties of adhesive resin and dental substrate. After forming an attachment between tooth and resin composite, resistance to fracture depends upon the extent of defect in the interface between bonding agent and tooth surface and there is the possibility of formation and widening of cracks and ultimately breaking of the attachment. This is also related to the total characteristics of the substrate, adhesive resin, and age of bonding.[1] The base of this adhesion is on the micromechanical retention that is proved particularly in the case of enamel, but is still a question about the dentin. In the present study, sound dentin was used as a substrate.

Previously, shear bond strength was the most common method used to examine the bond strength. In this method, a cutting force was applied by the tip of a blade to the interface between resin and dentin. The main problem of this method was the little attention paid to the geometry of the cavities and the shrinkage due to polymerization.[27]

MicroTBS is a relatively new method in evaluating bond strength that was introduced by Sano in 1994.[28] In this test, the bonding surface is decreased to approximately 1 mm^2. By reducing the bonding area, the defects are reduced to a minimum and the measured bond strength is near the actual and is higher than of tensile bond strength test. It is determined that a surface area of 1 mm^2 is critical in these tests and larger bond size causes higher than actual bond strength is recorded at greater bonding surface, while lower than actual bond is recorded when smaller area is tested.[29] Therefore, one of the standard criteria's in these tests is the surface area of about 1 mm^2 that was observed in this study. It is said that this test depicts a more realistic value of bond strength. Even though this method has several difficulties from the initial stage of gathering the specimens to implementation of the test, as the required sample size in this test is less than other tests, it would be one of the best methods to compare various types of bonding. Moreover, it is possible to recruit from different families of teeth. It is also possible to perform this test on dental surfaces with different clinical traits like dentinal caries, sclerotic dentin, and on the cervical region of root or enamel.[30] Samples with small defects may be excluded in order to get more realistic values of bond strength.[29] It is also possible to study the bond strength in different area of a single tooth.[31] Samples are more appropriate to be the subject of microTBS test than tensile bond strength test which needs more samples because the resin composite and surrounding dental tissue can protect the interface between tooth surfaces and resin composite from thermal changes.[15]

In order to perform microTBS test, samples usually are made in beam or hour-glass shape. In this study, hour-glass-shaped samples were used. The distance between pulp and interface between dentin and resin composite was equal or less than 3 mm. In addition, the upper and lower parts of hour-glass-shaped samples were stuck to the designed arms of testing machine and a larger surface area was prepared for attachment. This approach reduced the risk of early separation of the sample from the arm of testing machine. Certain studies, Nikaido *et al*.,[4] Bedran De castro *et al*.[10,11] Mitsui *et al*.,[5] Abdalla *et al*.[3] and Kasraei and Khamverdi,[8] used beam shaped samples in their studies, but Osorio *et al*.[32] and Toledano *et al*.[12] used hour-glass-shaped samples. Technique of microTBS test has its advantages and leads to higher accuracy in measurement over other methods, but it has its own several problems too, like preparing 1-mm thick slices and ultimately hour-glass or beam-shaped samples are highly technique-sensitive. In addition, placing the samples in the testing machine and fixing them is another sensitive step. If there is inadequate stability, the microtensile force will be altered and the results do not hold the required accuracy. Therefore, there is a risk of losing some of the samples during each stage of the study and a number of extra samples have to be prepared, as the backup, at the beginning of the study.

In the present study, diamond fissure burs were used to remove enamel and expose dentinal surface. In the study of Ogata *et al*.,[33] the effect of the type of bur on microTBS was evaluated. In that study, samples were obtained using various burs and results showed that the method of obtaining the smooth surface of dentin had no significant effect on microTBS, but the type of adhesive resin determined the value of bond strength.

In the present study, as the Kasrai *et al*. study,[8] the teeth were kept in normal saline and to prevent from cross contamination, 0.5% chloramine T solution was used. In the study of Zheng *et al*.,[34] the effect of the type of storage media on the bond strength of adhesive was studied. The storage media used in that study included distilled water at 4°C, 0.02% thymol, 10% formalin, 1% chloramine, and freezing of teeth at 20°C. They studied the bond strength of single adhesive resin to teeth and compared them with recently extracted teeth and concluded that storage media have a significant effect on the bond strength. They concluded that if a recently extracted tooth was not available, the best available method for storage of teeth would be 1% chloramine solution or freezing the teeth at 20°C (centigrade degree).[34]

In the present study, the maximum period of staying in storage media before study was one month. In the study of Miranda *et al*.,[35] the effect of storage duration on the bond strength was studied. It was concluded that the duration of staying in storage media before bonding has no significant effect on the bond strength and teeth can be kept for long periods of time in appropriate preservative mediums.

In the present study, the value of microTBS was measured with a cross head at the speed of 0.5 mm/min. In the study of Reis *et al*.,[36] the various speeds of cross head were evaluated. They showed that the differences between the effects of cross head cutting speeds of 0.5, 1, 2, and 4 mm/min on microTBS were not significant. However, cutting speeds of 0.5 mm/min and 1 mm/min have been used in most of the studies.

In the present study, the mode of fracture was evaluated by a stereomicroscope with ×20 magnification. Adhesive fracture was the most relevant mode of fracture in both the control group and test groups following mechanical and thermal load cycling, and is consistent with Bedran's study.[10,11] But, in Mitsui *et al*. study,[5] the most common mode of fracture was mixed and after increasing the thermal and mechanical load cycling the rate of this mode of failure increased. The reason of significant difference between the locations or types of fracture in studies is due to the variation in classification of fractures. Certain studies have reported that a vast number of cohesive fractures in dentin or adhesive resin is detectable by low magnification of stereomicroscope, but adhesive and mixed types of fracture are detectable only with high magnification.

The high rate of reported cohesive fractures in certain studies could be due to error in alignment of the position of samples in an examining machine or formation of small cracks during cutting that are mistakenly considered as cohesive fractures.[37]

In the present study, the mean value of microTBS decreased as the mechanical load cycling was increased and mechanical load cycling over 50K cycles caused a decrease in the value of microTBS and simultaneous use of mechanical and thermal load cycling decreased the values of microTBS.

The variation of results in different studies shows that there are several factors interfering with the generalization of the outcome of experiments to clinical trials. These factors include; type of teeth, storage media, infection control type, substrate and surrounding moisture content, presence or absence of thermo and or mechanical load cycling, depth, and location of selected substrate for the test, mechanical properties of the restorative substance, type of test (shear, micro shear, microtensile, and tensile), speed and magnitude of loading cross head strength, design, and dimensions of the final sample.[29]

CONCLUSION

Considering the limitations of the present experimental study, it can be concluded that, an increase in the mechanical load cycling, leads to a decrease in the value of microTBS but the minimum mechanical load cycles to make significant changes is 100K. Also, simultaneous application of thermal and mechanical load cycling decreases the value of microTBS and most of the fractures are of the adhesive type.

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