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Original Research

Effects of Thermal and Mechanical Load Cycling on the Dentin Microtensile Bond Strength of Single Bond-2

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Abstract:
Background: Different studies have shown the uncertain effects of thermal cycling (TC) and mechanical load cycling (MC) on the dentin microtensile bond strength (μTBS) of composites. This study designed to investigate the effects of TC and MC on the dentin μTBS of single bond-2.

Materials and Methods: Flat dentinal surface was prepared on 48 sound extracted human third molar teeth, and were bonded by single bond-2 adhesive and Z250 resin composite. The teeth were randomly divided into eight equal groups, according to the thermal/mechanical protocol. TC and MC were proceeded at 5-55°C and 90 N with 0.5 Hz. Then restorations were sectioned to shape the hour-glass form and subjected to μTBS testing at a speed of 0.5 mm/min. To evaluate the bonding failure, the specimens were observed under the scanning electron microscope. The results were statistically analyzed with analysis of variance, *t*-test, Tukey HSD and *post-hoc* by using SPSS software version 17 at a significant level of 0.05.

Results: μTBS of all groups were significantly lower than the control group ($P < 0.001$). Adhesive failure was predominant in all groups and increased with TC and MC.

Conclusions: TC and MC had an adverse effect on μTBS of the tested adhesive resin to dentin.

Key Words: Dentin bonding, mechanical cycling, microtensile bond strength, thermal cycling

Introduction

The demand for restoring teeth, not only anatomically and functionally, but also esthetically, has been increased in daily clinical practice.[1] Resin composites have been utilized remarkably in restorative dentistry to enhance dental structure or correction of tooth color.[1] A strong adhesion between tooth tissue and resin composite is essential for an ideal restoration. Enamel bonding has tremendous clinical success; however, dentin bonding cannot predictably be applied for long-term interfacial integrity. Since dentin is a vital part of the tooth and has complex biological structure,[2] it can influence the performance of different bonding strategies.

There are different techniques applied to create dentin bonding. Total-etch bonding systems work by removing the smear layer with phosphoric acid, applying a primer and adhesive in two different steps or even in the same step (total etch self-priming systems). The self-etching approach is another technique in which increased concentrations of acidic monomers enable the primer or adhesive to etch and prime the dentin simultaneously.[3]

In accordance with these systems, seven main generations of dentin bonding agents have been introduced in dentistry, but the fifth generation is more common for usage in adhesive treatments. In that generation, primer and bonding agent are combined into a single solution, so separate etching step is required.[4]

In clinical conditions, teeth are constantly encountered stresses during mastication and parafunctional habits.[5] These stresses will induce some micro cracks which subsequently jeopardize the long-term survival rate of bonding and final mechanical degradation of tooth tissue and restoration interface.[6] Several studies have shown that masticatory loadings could accelerate the degradation of dentin bonding interface.[1,7,8] The thermal cycles in oral environment can induce deteriorating stresses between a tooth substrate and a restorative material by generating expansion/contraction stresses.[1] Since impossibility of constant and rapid assessment of adhesive materials in clinical trial studies, use of thermal cycling (TC) and mechanical cycling (MC) would facilitate evaluation of dental materials similar to clinical conditions.[7,9,10]

The porosities and other internal defects within the adhesive layer may have detrimental effects on bonding durability. The static bond strength tests cannot adequately demonstrate it.[11] Cyclic loading could rapidly unfold the effect of these defects on long-term bonding.[10] Application of microtensile bond strength (μTBS) testing allows evaluation of the *in-vitro* bond strength of resin/dentin under more clinically relevant conditions than those which are usually performed in static bond strength testing techniques. Furthermore, this test enables researchers to measure bond strength at different regions.

Due to the limitations and difficulties of evaluating dentin/resin adhesion in clinical trial studies, researchers usually try to resemble oral environment *in-vitro* studies and attribute their results to actual conditions. The current study designed to evaluate the effects of TC and MC on μTBS of single bond-2 to dentin and observing the pattern of failure in bonded samples. The null hypothesis was that there would be no differences between μTBS of resin/dentin neither with nor without TC and MC.

Materials and Methods

Specimen preparation

In this *in-vitro* study, 48 freshly extracted intact and carries-free human third molar teeth, were collected and restored in distilled water at 4°C.[7] The teeth with any signs of cracks or developmental defect were excluded.

The teeth were cleaned and polished using slurry pumice with a brush and low-speed handpiece, and were disinfected by chloramine-T 1% (Merck, Darmstadt, Germany) solution for 24 h prior to testing.[12]

All of the samples were mounted in self-curing acrylic resin (Flash Acrylic, Yates Motloid, Chicago, USA) with the level of 1 mm below the cementoenamel junction (CEJ). Diamond burs (Teeskavan, Tehran, Iran) and high-speed handpiece with water spray were used to remove the occlusal enamel surface, beneath the pit and fissure surfaces, and flattened surface obtained with exposed underlying superficial dentin. Each bur was used for removing the enamel of five teeth. Before application of bonding agent, the dentin surfaces were treated by 320 grit silicone abrasive paper (Carbimet Discs, Buehler, Lake Bluff, IL, USA) for 15 s to create standard smear layer on the each tooth surface.[13]

Bonding procedure

In accordance with the instructions of single bond-2 (3M, ESPE, USA) company, the dentin surface of each specimen was acid-etched by 37% phosphoric acid gel for 15 s and then they were rinsed for 10 s by distilled water. The etched dentin was blot-dried according to wet bonding technique. Then single bond-2 was applied with micro brushes on the prepared dentin surface. Two layers of adhesive was applied and light cured by using light curing unit (Bluephase 20i, Vivadent, Lichtenestein, Germany) with a light intensity of 600 mw/cm^2 for 10 s. 3 mm Z250 resin composite (3M, ESPE, USA) in two layers were used to restore the bonded areas. The light curing distance was at a minimum distance for all of the samples, and each layer were light-cured for 40 s. All of the samples were mounted in self-curing acrylic resin with the level of 1 mm below the CEJ.

TC and MC procedure

Specimens were divided into eight equal groups (G1-G8), by random table number ($n = 6$). According to the TC and MC groups were as follow:

G1 (control): No thermo and MC; G2: 50 K MC; G3: 100 K MC; G4: 500 K MC; G5: 1000 TC; G6: 50 K MC+1000 TC; G7: 100 K MC+1000 TC; G8: 500 K MC+1000 TC.

For MC, teeth were subjected to load cycling (ERIOS, Sao Paulo, Brazil). 90 N force with a frequency of 0.5 Hz was applied.[14] The teeth were embedded in normal saline during MC.

The specimens from group G5 through G8 were thermocycled using a thermocycle machine (ERIOS, Sao Paulo, Brazil) at 5-55°C water baths with holding and dwell time of 60 and 15 s, respectively.

μTBS

The mounted teeth in self-cure acrylic resin were sectioned in mesiodistal direction and parallel to the vertical plane under running water to prepare two slabs with about 1 mm thickness from each tooth. The distance between pulp and dentin/resin composite interface was near 3 mm. A total of 12 samples were made in each group. The bonding area (0.8^{-1} mm^2 interface area) of each sample were thinned to create an hour-glass shape by using a diamond cylindrical bur (SS White, NY, USA). The upper and lower parts of hour-glass shaped samples were stuck to the designed arms of testing machine. Then, the samples were subjected to μTBS test by Universal Testing machine (Plus Universal Testing Machine MTD-500, Germany) at a crosshead speed of 0.5 mm/min in order to create fracture at the interface of composite and dentin.

Data analysis

The Kolmogorov–Smirnov test was used to evaluate the normal distribution of the groups. The data were analyzed by one-way analysis of variance (ANOVA), Tukey HSD and *post-hoc* tests. Two-way analysis variance was employed to determine the reciprocal effect of TC and MC on the μTBS, using SPSS 17 software program (SPSS 17, SPSS Inc., Illinois, Chicago, USA) at a significant level of 0.05.

In order to determine the location and mode of fracture, all of the samples were examined by using a scanning electron microscope (SEM) (HT 416002, Fuji, Japan).

Results

Kolmogorov–Smirnov test confirmed the normal distribution of data in all groups ($P = 0.322$).

Highest mean value in μTBS was in G1 (29.37 ± 2.89) and the lowest was in G8 (11.72 ± 2.76) (Table 1). Pairwise comparison among all groups was done by Tukey *post-hoc* test (Table 2) and the differences were significant ($P < 0.05$).

With an increase in the cycles of the mechanical load, either with or without TC, μTBS values decreased significantly ($P < 0.05$).

Furthermore, two-way ANOVA revealed that both TC and MC decreased μTBS and there was not a reciprocal difference between the effects of MC and TC ($P = 0.788$).

The type of fractures in all of the samples was studied by SEM. So, the maximum number of fractures was in adhesive (70.80%) and the minimum was in mixed types (3.20%) (Table 3).

Table 1: Results of two-way ANOVA test to compare μTBS means among studied groups.

MC	With thermocycling		Without thermocycling		*P* values
	Mean (MPa)	SD	Mean (MPa)	SD	
Control	26.64	2.53	29.37	2.89	0.022
50 K MC	21.43	2.92	23.90	1.87	0.022
100 K MC	19.89	1.49	21.42	1.86	0.038
500 K MC	11.72	2.76	13.56	1.23	0.048
P values	<0.001		<0.001		

MC: Mechanical cycle, SD: Standard deviation, μTBS: Microtensile bond strength, ANOVA: Analysis of variance

Table 2: Pairwise comparison (*P* values) of among studied groups.

Groups	G1	G2	G3	G4	G5	G6	G7	G8
G1	-	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.08
G2	-	-	0.14	<0.001	0/156	<0.001	<0.001	0.07
G3	-	-	-	<0.001	1.00	0.72	<0.001	<0.001
G4	-	-	-	-	<0.001	<0.001	0.51	<0.001
G5	-	-	-	-	-	0.71	<0.001	<0.001
G6	-	-	-	-	-	-	<0.001	<0.001
G7	-	-	-	-	-	-	-	<0.001
G8	-	-	-	-	-	-	-	-

Table 3: Distribution of the mode and failure of samples.

Groups	Adhesive (%)	Cohesive (%)	Mix (%)
G1	6 (50)	6 (50)	0 (0.0)
G2	8 (66.6)	4 (33.3)	0 (0.0)
G3	8 (66.6)	4 (33.3)	0 (0.0)
G4	9 (75.0)	2 (16.6)	1 (8.4)
G5	7 (58.3)	5 (41.7)	0 (0.0)
G6	10 (83.3)	1 (8.4)	1 (8.4)
G7	10 (83.3)	2 (16.6)	0 (0.0)
G8	10 (83.3)	1 (8.4)	1 (8.4)
Total	68 (70.8)	25 (26.0)	3 (3.2)

Discussion

A reliable and durable bonding of resin materials to dentin is important in the field of adhesive dentistry. An ideal study design to evaluate the quality of new bonding systems is a long-term clinical trial.[4] However, some difficulties cannot be overlooked such as Operator variability, substrate differences,[6] matter of time and resources,[6] etc. The current study was conducted to simulate clinical situations to evaluate the effects of TC and MC on the μTBS of Tetric N-bond to dentin. The results determined that the application of TC and MC simultaneously leads to decrease in μTBS. That result was in accordance to the studies by Mitsui *et al.*,[15] Abdalla *et al.*,[14] Toledano *et al.*,[3] Bedran-de-Castro *et al.*;[8] and in contrast with the result of De Munck *et al.*[9] and Nikaido *et al.*[10] Some factors play a determining role in the outcomes of μTBS test such as: Type of tooth and adhesive resin, the elapsed time of extraction, storage condition, composite resin type, mode of curing, intensity, and magnitude of applied force, number of TC and MC.[16] Intraoral restorations are consistently exposed to about 1 million (1000 K) mechanical strokes per year of the opposite tooth. These strokes might affect interfacial bonds of restoration and tooth surface, which may result in failure of the restoration. Many studies objected TC and MC techniques to provide conditions similar to the oral environment.[8] In the present study, mentioned methods used to mimic chewing condition, too. Different studies have reported different MC.[3,15,17] In the present study, the applied MC were 50 K, 100 K and 500 K, respectively.

Anderson[18] stated that the chewing and swallowing force is between 70 and 150 N. In the present study, the specimens were subjected to 90 N.

Plastic deformation of the adhesive interface and concentration of main stresses in hybridoid layer interface could be a possible explanation for present results in total-etch adhesive dentin bonding systems. Fatigue could be a facilitating factor for failure of bonding in hybridoid layer. Our results confirmed previous studies which indicated that fatigue could decrease resin-dentin bond strength.[15,17,19] Furthermore, it has been reported that demineralized dentin became weaker after cyclic loading.[20]

A study on a total-etch adhesive system showed that MC alone did not affect bond strength but when TC and MC were performed, bond strength decreased significantly.[8]

Based on the ISO TB 11450 standard, 500 cycles must be executed for TC. However, a review article reported that a TC of 10,000 cycles is similar to approximately 1-year fatigue in the oral environment.[9] In the present study, 1000 TC were used.

TC properly simulates oral condition in the laboratory.[21] This process helps to induce stress to a restoration due to aging, and thermal changes.[2] TC accelerates the hydrolysis of unprotected collagen fibers and removing the unpolymerized resin oligomers.[1,13,22] Higher amounts of thermal expansion and contraction of restorative materials in comparison to dental structures, might result in the formation of an interfacial gap. Furthermore, more stresses are induced in the higher ratio of configuration factor.[23] The effect of thermocycling on the bonding strength is not clear. One meta-analysis study showed that thermocycling had not a significant effect on the bond strength.[24]

It was reported that, higher μTBS values were presented in the alcoholic base adhesives after load cycling when the dentin was etched.[3] Existence of alcohol in a composition of adhesive can increase the infiltration in collapsed collagen network and improve tensile bond strength.[25] Hence, alcoholic base adhesive was used in the current study which created higher tensile bond strength.

μTBS is a relatively new technique for measuring bond.[26] Compared to other adhesive bond strength tests, μTBS test has several advantages, including the improvement of stress distribution during the test, the prevention of cohesive failures in dentin, the ability to measure regional differences in resin-dentin bond strength, and the ability to measure the higher bond strength of newly developed materials.[27] The measured bond strength would be near the actual and higher than tensile bond strength test.[16] Therefore, surface area of about 1 mm^2 is one of the standard criteria in these tests which was considered in this study. This test depicts more reliable values of bond strength, and it would enable comparing various types of bonding.[28]

On the other hand, μTBS test has its own several difficulties, like Required 1 mm thick slices, hour-glass or beam shaped samples, and its highly technique sensitive etc.[9]

Nikaido *et al.*,[10] Bedran-de-Castro *et al.*,[8,17] Mitsui *et al.*[15] and Abdalla *et al.*[14] used beam shaped samples in their studies, while Toledano *et al.*[3] used hour-glass shaped samples. In the present study, dentinal surface was exposed by diamond fissure burs. In a study by Ogata *et al.*,[29] the impact of the type of bur on μTBS was evaluated. In that study, samples were prepared by using various burs and final results showed no significant differences between burs, but the bond strength values were differed when different types of adhesive resin were used.

In this study, the value of μTBS was measured by using a cross head at the speed of 0.5 mm/min. In a study by Reis *et al.*,[30] the effect of various cross head speed on μTBS were observed, and no significant differences were found among 0.5, 1, 2 and 4 mm/min. However, cutting speeds of 0.5 and 1 mm/min have been used in several studies. In the present study, fracture mode was evaluated by SEM and stereomicroscope with ×40 magnification. Adhesive fracture was the most relevant fracture mode in both control and test groups which was in accordance with the Bedran-de-Castro *et al.* studies.[8,17] But, in Mitsui *et al.* study[15] the most common fracture mode was in a mixed pattern and after increasing the TC and MC, the rate of this failure pattern was increased. Variation in classification of fractures might be the reason of different locations or types of fracture in different studies. Certain studies have reported that vast numbers of cohesive fracture were detectable by low magnification stereo microscope, but adhesive and a mixed pattern of fracture would be detectable only in high magnification.

The high rate of reported cohesive fractures in some studies could be due to misalignment of the samples' position, formation of small cracks during slicing that could be mistakenly considered as cohesive fractures.[31]

Variation of results in different *in-vitro* studies interfere with the generalized conclusion in clinical experiments. This variation might be due to: teeth type, storage environment, control infection condition, presence or absence of TC and/or MC, mechanical properties of the restorative materials, type of test (shear, micro shear, micro tensile, and tensile), speed and magnitude of the cross head, design and shape of the final sample.[16]

Conclusions

With limitation of the present study, it was shown that TC and MC had an adverse effect on μTBS of Tetric N-Bond adhesive to dentin.

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