

An Alternative Method for Thermal Cycling Test: Effect on the Marginal Microleakage and Bond Strength of Dental Polymer Bonded to Dentin

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This study evaluated an alternative method for thermal cycling test on the microleakage and bond strength of the polymer-dentin bond. For the microleakage test the cavities were restored with a TEGDMA+UDMA+bis-EMA composite polymer light cured for 20 s. Samples were immersed in 2% methylene blue solution for 2 h and sectioned. Microleakage scores were submitted to Kruskal-Wallis test. For the shear bond strength test the adhesive was applied to dentin, photoactivated for 10 s and the composite polymer incrementally photoactivated. Samples were submitted to shear bond strength test in a machine with a cross-head speed of 0.5 mm/min and data were submitted to ANOVA and Tukey's test. Studied groups were: 1 - without thermocycling; 2 - thermocycled at 5 °C and 55 °C with intermediate bath at 37 °C; 3 - thermocycled at 5 °C and 37 °C; 4 - thermocycled at 37 °C and 55 °C; 5 - thermocycled at 5 °C and 55 °C (traditional test). Cold baths promoted greater microleakage when compared to control and hot bath, whereas control and hot bath were similar. Cold baths presented significant lower shear bond strength than those submitted to hot bath and control. It was concluded that the alternative method for thermal cycling test showed that cold temperatures increased the microleakage and decreased the bond strength of the polymeric adhesive.

Keywords: dentin bond strength, marginal microleakage, thermocycling temperatures

1. Introduction

Previous studies has shown that the method of increasing the adhesion of polymeric filling materials (organic matrix and inorganic filler) to tooth surfaces was possible due to the alteration of the tooth surface by chemical etching to produce a roughness surface to which the polymeric materials might adhere¹ and to the development of polymeric materials which have adhesive properties²⁻⁴. Consequently, this technical possibility was the initial step for the establishment of esthetic restorations with improved marginal integrity and bond strength⁵⁻⁷.

The presence of a conditioned dentin zone where occurs the polymeric infiltration has also been demonstrated in classic studies^{3,8,9} and other more recent investigations has focused the importance of the polymer-dentin interdiffusion zone in the adhesive restorations with dental composites¹⁰⁻¹². This zone is characterized by the diffusion of different adhesive systems through inter and peritubular dentin, improving the marginal sealing and the bond strength between dental polymer and tooth^{3,13}.

The bond quality of the polymeric adhesives to the tooth is often verified in laboratories by tests involving shear or tensile bond strengths, marginal microleakage or gap formation almost always with some technical limitation^{4,14-16}.

In addition, it has shown that comparisons among in vitro tests are difficult even in similar studies. The obtained values depend on the type and details of the test method used, type and quality of dentine, sample storage conditions prior to testing, quality of the involved material and how it is handled^{4,17}.

Despite the clinical trials be considered essential to study the long term behavior of adhesive systems, laboratory tests could provide a fast mechanism to understand, clarify and compare the probable effect of several systems^{10,17}. Although many laboratory studies can not be directly related to clinical situations, they are always beneficial to provide an acceptable degree of reliability for the establishment of clinical studies on adhesive of polymeric systems used in enamel and dentin.

The International Standardization Organization (ISO) published a guide for the testing of adhesive materials to tooth structures in order to standardize, as far as possible, the different methods of evaluation, the quality of dental materials and its tooth structure bonds¹⁸. According to ISO specification¹⁸, marginal microleakage test should be preceded by the samples thermocycling in water bath temperatures at 5 °C and 55 °C to simulate the thermal changes occurred in the oral environment.

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Effects of these thermal changes on the different thermal expansion coefficients of the dental polymer and tooth structures may be critical for bond durability. To date, however, no report in the literature has shown which temperature level should be more harmful to the maintenance of the dental polymer-tooth structure adhesive bonding, since the thermocycling test involves association of thermal changes between cold and hot water baths.

Considering the uncertainty regarding the thermal changes occurring in oral environment, this study was conducted to evaluate alternative methods for the traditional thermocycling test (cycling at 5 °C/55 °C) on the marginal microleakage and shear bond strength of dental polymer bonded to bovine dentin in Class V cavities. The hypothesis of this work should be that both alternative and traditional thermocycling methods should promote similar effect on the bonding strength and marginal microleakage between dental polymer and bovine dentin.

2. Material and Methods

2.1. Materials

Filtek Z250 composite polymer (3M ESPE, St. Paul, MN, USA; basic composition: BIS-EMA, Bis-GMA, UDMA, zircona/silica particles; batch # 3AM) and Adper Single Bond adhesive system (3M ESPE; basic composition: water, alcohol, HEMA, Bis-GMA, dimethylacrylates, photoinitiator system and copolymer from the polyacrylic and polyitaconic acids: batch # 2GU) were used in this study.

2.2. Microleakage test

Fifty bovine mandible incisor teeth were selected. The teeth were cleaned thoroughly with pumice slurry and stored for one week in deionized water at room temperature. Standardized Class V cavities were prepared in all samples using a handpiece (Dabi Atlante, Ribeirao Preto, SP, Brazil) with a FG 3053 spherical diamond bur (KG Sorensen, São Paulo, SP, Brazil) with 2 mm-diameter and 126 to 91 µm-diamond particles grid. All cavities were prepared with enamel margin of 4 mm in diameter and 2 mm in depth. The diamond bur was replaced after five cavity preparations.

All cavity surfaces were etched with 35% phosphoric acid gel (3M ESPE) according to the manufacturer's instructions (application for 15 seconds and water rinse for 30 s). The water excess of the cavity rinsing was lightly removed with hydrophilic cotton pellets before application of 2 consecutive layers of Adper Single Bond (3M ESPE) on the dentin. Before the photoactivation of the two adhesive layers for 10 s each one, the first layer was lightly dried with a stream of air.

Filtek Z250 composite polymer was applied in one increment and photoactivated using a halogen lamp unit (XL 2500, 3M ESPE) with light intensity of 700 mW/cm² for 20 s. Finishing and polishing of the Class V restorations was performed using water cooled fine and ultra fine Sof-Lex disks (3M ESPE), 24 h after the composite resin filling procedures.

Samples were randomly assigned into 5 groups (n = 10), according to the temperatures of the thermocycling cycles:

1- without thermocycling (control); 2- intermediate thermocycling: 1,000 cycles at 5 °C and 55 °C, for 30 s each one, with intermediate water bath at 37 °C, for 30 s; 3 - cold thermocycling: 1,000 cycles with water bath at 5 °C and 37 °C, for 30 s each one; 4 - hot thermocycling: 1,000 cycles with water bath at 37 °C and 55 °C, for 30 s each one; 5- traditional thermocycling: 1,000 cycles at 5 °C and 55 °C, for 30 s each one. Samples thermocycling was performed in a thermal cycling machine (MSCT-1, São Carlos, SP, Brazil).

All samples were coated with two layers of nail polish (Revlon, São Paulo, SP, Brazil) up to 1 mm from the marginal line of the cavity. Following, the samples were immersed in a freshly prepared aqueous 2% methylene blue buffered solution (pH 7.0) for 2 h at room temperature.

After dye immersion, the samples were washed in running water and the nail polish seal removed. Each sample was sectioned vertically through the center of the Class V restoration, from mesial to distal, in a cutting machine (SBT-Model 650, San Clement, CA, USA) with a diamond disc (Extec Corp., Enfield, CT, USA) at low speed under water cooling. Afterwards, the samples were polished with 1,500 grit silicon carbide paper (Norton, Sao Paulo, SP, Brazil) cooled with water, in an automated rotary polishing unit (Arotec, São Paulo, SP, Brazil).

The microleakage evaluation was made in the two portions of the sectioned sample using an optical stereomicroscope (Carl Zeiss, Gottingen, Germany) at 70 × magnification. The mean scores were determined by one operator, which did not know who was the group of the samples, using the following criterion: 0- no dye penetration; 1- dye penetration in enamel; 2- dye penetration beyond the dentin-enamel junction, without reaching the axial wall; and 3- dye penetration in the axial wall.

The evaluation and quantification of the marginal dye microleakage was based on the ISO/TS 11405 guidance¹⁸. Obtained data were submitted to the Kruskal-Wallis non-parametric test (5%).

2.3. Shear bond strength test

Fifty bovine mandible incisor teeth were used following selection, cleaning and storage, as described previously for the microleakage test. The teeth roots were sectioned and the crowns embedded in chemically-activated acrylic resin (Classico Dental Products, Sao Paulo, SP, Brazil) positioned in 20-mm diameter and 20-mm length PVC tubes (Tigre Plastic Manufacturer, Criciuma, SC, Brazil), with the buccal face positioned 1 mm beyond the edge of the tube. Teeth buccal faces were ground and polished using 180, 400 and 600 grit sandpaper (Norton, Guarulhos, SP, Brazil) in an automated rotary polishing unit (Arotec). After this procedure, the abraded dentin surface was examined under 60 × magnifications in an optical stereomicroscope (Carl Zeiss, Germany) to verify the dentin conditions. An area of 4 mm in diameter was delineated in the prepared dentin with adhesive tape (Contact, São Paulo, SP, Brazil) for standard the bonding area of the dental polymer.

After dentin acid etching for 15 s, the samples were washed for 30 s, and the water excess gently removed with pellets of hydrophilic cotton. The samples were single positioned in a circular metallic die and maintained in

position by means of a screw. Two layers of Adper Single Bond adhesive system (3M ESPE) were applied to dentin. Before the photoactivation of the two adhesive layers for 10 s each one, the first layer was lightly dried with a stream of air. Afterwards, three layers of Filtek Z250 composite polymer (3M ESPE) were incrementally placed inside the metallic die on the etched dentine surface according to manufacturer's instructions and each layer photoactivated with a XL2500 unit (3M ESPE), with intensity of 700 mW/cm² for 20 s.

The samples were randomly assigned into 5 groups (n = 10), stored in relative humidity inside an oven (ECB 1.1, São Paulo, SP, Brazil) at 37 °C for 24 h, and submitted to the same thermocycling protocol as described in the microleakage test. Samples were submitted to the shear bond strength test in an universal machine (Instron Co., Canton, MA, USA), with a cross-head speed of 0.5 mm/min. Each sample was horizontally placed in a metallic glove (20.5 mm internal diameter by 20 mm height) fastened to the superior cramp of the machine. During the applied tensile load, the ends of a stainless steel tape (5 mm in width and 10 cm in length) were fastened in the inferior mordant forming a loop that enclosed the composite cylinder bonded to the dentin, resulting in shear bond strength at the dental polymer-tooth interface.

Shear bond strength value in kgf/cm² was calculated by the following formula: $RC = F/A$, where RC is the shear bond strength, F is the applied load, and A is the bonded area. The values in kgf/cm² were converted in MPa multiplying by the constant 0.098. Collected data were submitted to one-way ANOVA and Tukey's test at a significance level of 5%.

3. Results

3.1. Microleakage test

Table 1 shows the marginal microleakage values for the medium posts among groups and the resulting analysis by the Kruskal-Wallis non-parametric test. Groups submitted to cold thermocycling baths (5 °C and 55 °C with intermediary bath at 37 °C; 5 °C and 37 °C or 5 °C and 55 °C) were greater and statistically different from the control and hot thermocycling bath groups (37 °C and 55 °C). Control and hot thermocycling groups were similar.

3.2. Shear bond test

Samples submitted to thermocycling at 5 °C and 55 °C with 37 °C intermediary bath, at 5 °C and 37 °C or at 5 °C and 55 °C presented statistically significant lower shear bond strength values than those submitted to both hot water bath (37 °C and 55 °C) and control groups (Table 2).

3.3. Failure mode

Failure mode of the fractured samples was evaluated under 40 × magnifications in an optical microscope (Carl Zeiss, Germany). Debonded surface zone showed that the failure was predominantly interfacial between polymeric adhesive and dentin in the cold cycling groups, which presented lower shear bond strength values when compared to the hot cycling group. In both control and hot cycling groups, the failure more commonly observed was mixed (adhesive and cohesive in the adhesive layer).

Table 1. Medium posts comparison among groups for marginal microleakage.

Thermocycling group	Medium post
Control (without cycling)	17.90 ^b
Cycling at 5 °C/37 °C/55 °C	32.30 ^a
Cycling at 5 °C/37 °C	31.50 ^a
Cycling at 37 °C/55 °C	12.30 ^b
Cycling at 5 °C/55 °C	33.50 ^a

Medium post followed by different letters show statistically significant difference by the Kruskal-Wallis test (5%).

Table 2. Mean values for shear bond strength (MPa) and SD for thermocycling groups.

Thermocycling group	Mean (MPa)
Control (without cycling)	6.49 ± 2.58 ^a
Cycling at 5 °C/37 °C/55 °C	4.03 ± 1.87 ^b
Cycling at 5 °C/37 °C	4.01 ± 0.92 ^b
Cycling at 37 °C/55 °C	6.41 ± 2.31 ^a
Cycling at 5 °C/55 °C	3.95 ± 1.14 ^b

Means followed by different letters show statistically significant difference by the Tukey's test (5%).

4. Discussion

Although the bond integrity may be established during or after composite resin polymerization, marginal leakage can occur afterwards in clinical use due to chemical, thermal and mechanically conjugated stresses occurring in the adhesive interface¹⁹⁻²¹.

Thermal cycle procedure is often employed in laboratory studies to evaluate the dental marginal sealing^{4,14,15,17}. However, it is difficult to establish a relationship between different studies, since there is always some variation concerning the temperature levels used in the thermal water baths, amount of cycles and immersion times in each bath¹⁵. In addition, the filling material used and the cavity preparation type may also be different and to promote different results among similar studies.

Table 1 shows that the group submitted to temperature at 37 °C and 55 °C presented lower penetration of dye, with similar value to the control group. In these conditions, this result shows that the high temperatures promoted small damage in the adhesive bond quality between dental polymer-tooth structures.

On the contrary, the groups submitted to thermal cycle with water baths at 5 °C and 55 °C with 37 °C intermediary bath; 5 °C and 37 °C or 5 °C and 55 °C showed greater values of dye infiltration, which were significantly different from the hot water bath (37 °C and 55 °C) and control groups, both with smaller results.

The thermal cycle may induce some changes at the composite polymer-adhesive-dentin interface due to differences in the coefficients of linear thermal expansion between adhesive and tooth structure²¹. This fact can suggest that different stresses induced by different temperatures used in thermal cycles are more related to the polymeric adhesive materials than the tooth substrates available to adhesion. In the present study, the adhesive bond rupture was more

critical when the sample was submitted to thermal cycle at lower temperatures, since the microleakage was greater in the samples submitted to the cycles with cold baths (5 °C). In addition, it is possible to suppose that the shrinkage for both polymer and tooth structure was more significant at lower temperature, causing obviously more adhesive failures. It is possible to infer that the resulting shrinkage was greater in the groups submitted to thermal cycle with cold temperature. Consequently, the probability for gap formation was great due to dimensional change to occur in the same direction, but with opposite effects on the polymeric materials (contraction/expansion). Conversely, the samples submitted to association of cold and hot temperatures presented gaps probably with similar sizes. An absence of volumetric compensation between contraction and expansion values is a supposition that could explain these results. If so, the expansion magnitude promoted by the hot temperature was not sufficient to balance the contraction magnitude occurred in the cold temperature. In addition, the hot cycle promoted smaller expansion, maintaining the adhesive bond in similar condition to those of the control samples. Probably, this fact was due to the dimensional changes occurred in same direction (expansion) and its consequent effects on the adhesive interface.

Results of this study showed that the cold thermal cycles may be responsible for the decreased adhesive bond strength between composite polymer and tooth. This fact is due to the thermal shock promoted by the association of cold and hot temperatures on the adhered structures.

It is claimed that samples must be submitted to traditional thermal cycle between 5 °C and 55 °C to simulate the thermal changes occurring in oral conditions¹⁸. However, the literature does not determine which temperature may cause more damage to dental polymer-tooth bond, considering that thermal cycle test involves cold and hot temperatures. In addition, it is difficult to compare several studies performed with different temperature of immersion baths, different cycle numbers, immersion times in each bath, and the use or not of intermediary baths.

Table 2 shows that both control and hot thermal cycle (37 °C and 55 °C) groups presented the greatest shear bond strength values. This result showed that the highest temperatures promoted smaller damage to the dental polymer-tooth bond. The samples submitted to thermal cycles at 5 °C and 55 °C with 37 °C intermediate bath; at 5 °C and 37 °C; and at 5 °C and 55 °C presented shear bond strength values with statistically significant difference when compared to those submitted to the hot water bath cycle (37 °C and 55 °C) and control group.

In the current study, the failure of the bond was more critical when the samples were submitted to thermal cycles with lower temperatures, since the shear bond strength was lower in the samples submitted to cold temperature (5 °C). These groups showed the lower shear bond strength values when compared to the hot cycling groups. Table 3 shows that the fracture was predominantly adhesive (between material and bovine dentin) in all cold cycling groups. This fact probably occurred in function of the greater contraction occurred in the material and tooth structure

Table 3. Predominant failures for samples submitted to shear bond strength test.

Thermocycling group	Predominant failure
Control (without cycling)	Mixed
Cycling at 5 °C/37 °C/55 °C	Adhesive
Cycling at 5 °C/37 °C	Adhesive
Cycling at 37 °C/55 °C	Mixed
Cycling at 5 °C/55 °C	Adhesive

at this temperature, causing more adhesive failure for the same reasons as described for the microleakage marginal results. The cold thermal cycle could be responsible for the decrease of the bond strength between tooth and adhesive, with similar conditions of thermal shock that occurs when the cold and hot temperatures are associated. Conversely, in the hot cycling groups the failure more commonly observed was mixed (adhesive and cohesive in the adhesive layer), as showed in the Table 3.

The hypothesis of this study that both alternative and traditional thermal cycles should promote similar effect on microleakage and shear bond strength of composite polymer bonded to dentin was not confirmed. Based on these conditions, it is possible to presume that the bond between adhesive and dental composite is differently influenced by the temperature levels due to the intrinsic properties of each material and dental structures involved in the process.

It is important to consider that the coefficient of linear thermal expansion (CLTE) of the composite dental polymer is different from the tooth structure. In addition, the CLTE of the commercial composite resins depends on their chemical structure and the thermomechanical analysis depends mainly on the inorganic filler content and also on the chemical structure of the organic matrix-resin²². Based on this consideration, when the amount of organic matrix is greater, the linear expansion of the composite resins is also greater. Inversely, a larger amount of inorganic filler decreases the linear expansion of the dental composite polymers (to a similar volume of a same material). According to Anusavice²³, the linear thermal expansion coefficient of composite dental polymers ranges from 14 (more inorganic filler content) to 50 (more organic matrix content), whereas the coefficient of the enamel is 11,4 and of the dentin is 8.3.

From this observation, cold temperature in oral environment should be more harmful to dental structures and restorative composite materials than hot temperatures. This study did not evaluate possible concepts related to the substrates to explain the results obtained, as diffusibility, conductivity, and coefficient of thermal expansion of the involved structures. However, this alternative thermal cycle test may verify the effects that the cold temperature may have on other variables clarifying their mechanical behavior. These aspects would be an area for future studies focusing on composition of polymeric adhesives and dental composite polymer, types of photoactivation units and photoactivation methods by modulation. Moreover, other studies focusing on thermal cycling could benefit from the proposed method.

5. Conclusion

- Microleakage values showed by alternative cold thermocycling baths (5 °C/37 °C/55 °C and 5 °C/37 °C) and traditional bath (5 °C/55 °C) were greater and statistically different from the control and hot thermocycling bath (37 °C/55 °C). Control and hot bath were similar;
- Cold thermocycling baths (5 °C/37 °C/55 °C and 5 °C/37 °C) and traditional bath (5 °C/55 °C) presented statistically significant lower shear bond strength values than those submitted to both control and hot water bath (37 °C and 55 °C);

- In the cold cycling groups the failure was predominantly interfacial between polymeric material and dentin. The failure more commonly observed in control and hot cycling groups was mixed (adhesive and cohesive in the adhesive layer); and
- From these observations, cold temperature in oral environment should be more harmful to dental structures and restorative composite materials than hot temperatures.

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