Universidade Estadual de Campinas
Faculdade de Odontologia de Piracicaba

Ana Karina Barbieri Bedran de Castro

INFLUÊNCIA DAS CICLAGENS TÉRMICA E MECÂNICA NA ADESÃO À ESTRUTURA DENTINÁRIA

Tese apresentada à Faculdade de Odontologia de Piracicaba, da Universidade Estadual de Campinas, para obtenção do título de Doutor em Clínica Odontológica, área de concentração Dentística.

Piracicaba
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Cirurgiã-Dentista

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A Comissão Julgadora dos trabalhos de Defesa de Tese de DOUTORADO, em sessão pública realizada em 22 de Abril de 2003, considerou a candidata ANA KARINA BARBIERI BEDRAN DE CASTRO aprovada.

1. Prof. Dr. LUIZ ANDRE FREIRE PIMENTA________________

2. Prof. Dr. RENATO HERMAN SUNDFELD________________

3. Prof. Dr. PAULO EDUARDO CAPEL CARDOSO________________

4. Prof. Dr. MARCELO GIANNINI________________

5. Prof. Dr. MARIO ALEXANDRE COELHO SINHORETI________________

Dedico este trabalho a minha família,

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Uma vez estabelecida a integridade da união durante e logo após a polimerização da resina composta, o desenvolvimento da infiltração marginal pode ocorrer depois de algum tempo devido ao estresse químico, térmico e/ou mecânico na interface adesiva. Sendo assim, avaliações "in vitro" empregando ciclos térmicos e mecânicos possibilitariam a simulação de alguns desafios clínicos aos quais diferentes materiais restauradores e substrato dentinário seriam expostos. Este estudo "in vitro" teve como objetivo estabelecer a influência da ciclagem térmica e mecânica através de cinco metodologias de avaliação: 1- Microinfiltração marginal de restaurações de resina composta em cavidades classe II do tipo "slot" vertical, com margem em dentina, através da penetração de corante ; 2- Resistência da união entre o substrato dentinário e o material restaurador, por meio do teste de cisalhamento; 3- Resistência a microtração da margem cervical de restaurações de resina composta em cavidades classe II do tipo "slot" vertical, com margem em dentina; 4- Avaliação de nanoinfiltração de restaurações de resina composta em cavidades classe II do tipo "slot" vertical, com margem em dentina; e 5- Durabilidade da resistência a microtração da margem cervical de restaurações de resina composta em cavidades classe II do tipo "slot" vertical, com margem em dentina. De acordo com os estudos desenvolvidos, pode-se observar que para a avaliação da microinfiltração, utilizando azul de metileno, nenhum efeito da ciclagem térmica e ciclagem mecânica, bem como a associação de ambas, foi observado. Para avaliação da resistência ao cisalhamento, nenhum efeito dos diferentes tipos de estresses (térmico e mecânico) foi observado. No entanto, para as avaliações de nanoinfiltração e de microtração, a associação de ciclagem térmica e mecânica resultou em significante aumento na extensão de penetração de nitrato de prata e diminuição nos valores de resistência a microtração. Diminuição dos valores de adesão foi observada após um ano de estocagem, onde estas diferenças não foram relacionadas ao uso de ciclagem térmica e mecânica.

Once the bonding was achieved during and after composite resin polymerization, the development of marginal leakage can occur after a long period of time, due to chemical, thermal, and mechanical stresses. In this way, "in vitro" evaluations using thermal cycling and mechanical load cycling would allow simulations of oral challenges, in which material and tooth substrate are exposed. This "in vitro" study aimed to established the influence of thermal and mechanical load cycling on five methodologies of evaluation: 1- Marginal microleakage of class II type box restored with composite resin, with cervical margin at dentin; 2- Bond strength between flat dentin substrate and restorative material, using shear bond strength test; 3- Microtensile bond strength of cervical margins of class II box type restored with composite resin, with margin in dentin; 4- Nanoleakage evaluation of class II composite resin restorations with cervical margin in dentin; and 5- Long term evaluation of the bond strength of cervical margins of class II box type restored with composite resin, with margin in dentin. According to the different studies performed distinctly, it was observed that for microleakage, using methylene blue, no effect of thermal cycling, mechanical load cycling, and thermal and mechanical load cycling performed in the same specimen was related. For shear bond strength, no effect of thermal and mechanical load cycling performed alone or together was related. On the other hand, for nanoleakage and microtensile bond strength evaluations, a statistically significant effect on respectively, increase in length of penetration and decrease in bond strength was observed when thermal cycling and mechanical load cycling were performed in the same specimen. Long term bond strength evaluation resulted in a significantly decrease in bond strength after 1 year, where the decrease was not related to the use of thermal and mechanical load cycling.

INTRODUÇÃO

O desenvolvimento de materiais adesivos, associado aos conceitos de prevenção e promoção de saúde, permitiram a evolução da odontologia restauradora, baseada na extensão, para prevenção associada ao uso de formas de retenção e resistência; para uma odontologia baseada na manutenção de estrutura dental hígida (ABDALLA & DAVIDSON, 1993; BARATIERI *et al.*, 1998).

Para o sucesso do tratamento restaurador adesivo, um dos principais objetivos é o selamento efetivo da restauração realizada; e em ordem para assegurar que o dente restaurado não sofra infiltração, a interface dente/restauração deve ser capaz de resistir às tensões geradas pelas alterações dimensionais (LEIBROCK *et al.*, 1999). Dentre os materiais utilizados para realização de restaurações adesivas, dois destes contribuíram para a extensa aplicação em dentes posteriores: as resinas compostas e os sistemas adesivos.

O primeiro desafio para manutenção da integridade das restaurações de resina composta é o controle da reação de polimerização, (FERRACANE & CONDON, 1999) que promove uma contração de 2,7 a 5,6% do seu volume (FEILZER, DE GEE, DAVIDSON, 1988). Esta contração é responsável por forte tensão na interface, podendo ocasionar rompimento de união e consequentemente a desadaptação do material na cavidade, levando a um processo de infiltração marginal, podendo levar também ao desenvolvimento de cárie secundária e sensibilidade pós-operatória (ABDALLA & DAVIDSON, 1993; ALANI & TOH, 1997; DIETSCHI & HERZFELD, 1998; MIYAZAKI *et al.*, 1998).

O constante aprimoramento das resinas compostas, através da incorporação de diferentes partículas de carga (BOWEN, 1963), modificações na quantidade e tipo destas partículas (EHRNFORD, 1981), emprego de diferentes formas de inserção e métodos de polimerização (DARBYSHIRE, MESSE, DOUGLAS, 1988; LOESCHE, 1999), contribuíram para melhorar as propriedades das resinas compostas; possibilitando o seu uso em dentes posteriores.

Paralelamente à evolução das resinas compostas, o desenvolvimento dos sistemas adesivos está intimamente relacionado com o sucesso do tratamento restaurador. Com a técnica do condicionamento ácido total (FUSAYAMA *et al.*, 1979) e o desenvolvimento dos sistemas adesivos hidrófilos (NAKABAYASHI, 1985), altos valores de resistência de adesão e selamento marginal foram encontrados. Atualmente, os estudos avaliando adesivos tem se direcionado para a adesão à dentina, uma vez que tem sido relatado ser menos efetiva do que em esmalte

(McCAHREN *et al.*, 1990; YAP, STOKES AND PEARSON, 1996; MIYAZAKI *et al.*, 1998; PERDIGÃO & LOPES, 1999) e também à simplificação da técnica adesiva, através do emprego de sistemas de frasco único e sistemas "auto condicionantes" (YAP, STOKES, PEARSON, 1996; CARDOSO *et al.*, 1999).

Uma vez estabelecida a integridade de união durante e logo após a polimerização da resina composta, o desenvolvimento da infiltração marginal pode ocorrer depois de algum tempo devido ao rompimento da união pelo estresse químico, térmico e/ou mecânico na interface adesiva (JORGENSEN *et al.*, 1985; ABDALLA & DAVIDSON, 1993; ABDALLA & DAVIDSON, 1996; DAVIDSON & ABDALLA, 1994; MELLO *et al.*, 1997), e desta maneira, as aplicações de diferentes formas de estresse são incluídos nos delineamentos experimentais "in vitro" a fim de submeter a restauração a um forte estresse (MELLO *et al.*, 1997).

A aplicação de metodologias avaliando a capacidade seladora de diferentes materiais, bem como a sua resistência de união são extensivamente empregadas em estudos "in vitro" com o objetivo de avaliar o comportamento do material.

O uso de ciclos térmicos é freqüentemente incluído nos estudos laboratoriais avaliando selamento marginal (KIDD, 1976; ALANI & TOH, 1997; DARBYSHIRE, MESSER, DOUGLAS, 1988; YAP, STOKES, PEARSON, 1996; YAP, 1997; DIETSCHI & HERZFELD, 1998; HAKIMEH *et al.*, 2000), sendo também utilizado para estudos avaliando resistência da união (LEIBROCK *et al.*, 1999). Sua aplicação tem sido questionada quanto a real efetividade no desenvolvimento de estresse e conseqüente alteração dimensional na interface dente restauração (KIDD, 1976; ALANI & TOH, 1997; MIYAZAKI *et al.*, 1998; HAKIMEH *et al.*, 2000). No entanto, torna-se difícil correlacionar os diferentes estudos, pois existe variação em relação ao emprego das temperaturas dos banhos de imersão, a quantidade de ciclos, o tempo de imersão em cada banho e a presença de banhos intermediários.

Foi sugerido que a aplicação de carga oclusal também pode promover alterações na interface dente/restauração (ABDALLA & DAVIDSON, 1996; DAVIDSON & ABDALLA, 1994; DELONG & DOUGLAS, 1983; DIETSCHI & HERZFELD, 1998; MELLO *et al.*, 1997). Estudos avaliando infiltração marginal (Abdalla & Davidson, 1996; Davidson & Abdalla, 1994; DELONG & DOUGLAS, 1983; HAKIMEH *et al.*, 2000; HTANG, OHSAWA, MATSUMOTO, 2000; LUNDIN & NOREN, 1991; MUNKSGAARD & IRIE, 1988; YAP, 1997) e resistência adesiva (LEIBROCK *et al.*, 1999; MCCAGHREN *et al.*, 1990; WILLIAMSON, MITCHELL, BREEDING, 1993) têm incluído o uso de ciclagem mecânica nos delineamentos experimentais. Entretanto, assim como para a ciclagem térmica, existe grande dificuldade em comparar os diferentes estudos, devido as diferencas na quantidade de ciclos, na força exercida e na freqüência em que é desenvolvida.

O estabelecimento de metodologias empregando diferentes formas de estresse se faz muito importante (LEIBROCK *et al.*, 1999), uma vez que a rápida evolução tanto dos sistemas adesivos como das resinas compostas não permite estudos clínicos de longa duração. Sendo assim, avaliações "in vitro" empregando ciclos térmicos e mecânicos possibilitariam a simulação de alguns desafios clínicos aos quais diferentes materiais restauradores e substratos dentinários seriam expostos (DAVIDSON & ABDALLA, 1994; LEIBROCK *et al.*, 1999; MELLO *et al.*, 1997).

Este estudo "in vitro" teve como objetivo estabelecer a influência das ciclagens térmica e mecânica :

- Na avaliação de microinfiltração marginal de restaurações de resina composta em cavidades classe II do tipo "slot" vertical, com margem em dentina, através da penetração de corante ;
- Na resistência da união entre o substrato dentinário e o material restaurador, por meio do teste de cisalhamento;
- Na resistência a microtração da margem cervical de restaurações de resina composta em cavidades classe II do tipo slot vertical, com margem em dentina;
- Na avaliação de nanoinfiltração de restaurações de resina composta em cavidades classe II do tipo slot vertical, com margem em dentina; e
- Na durabilidade da resistência de união de restaurações de resina composta, com margem em dentina, estocadas por um período de um ano.

CAPÍTULO 1

INFLUENCE OF THERMAL AND MECHANICAL LOAD CYCLING ON MICROLEAKAGE AND SHEAR BOND STRENGTH TO DENTIN

Short title: Thermal/mechanical cycling on leakage/bond strength

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Clinical significance: *The simulation of thermal and mechanical stresses presented in the oral environment does not interfere with microleakage and bond strength values.*

Summary

The purpose of the study was to evaluate the influence of mechanical and thermal cycling on microleakage at the cervical margins of proximal slot restorations and shear bond strength on flat dentin surfaces. Microleakage evaluation - 120 slot cavity restorations were performed on bovine incisors. The restorations were randomly divided in 4 groups (n=30): control (no thermal and mechanical load cycling), thermal cycling (2,000 cycles, 5-55°C), mechanical load cycling (50,000 - 80N), thermal and load cycling (2,000 5-55°C/ 50,000 - 80N). The specimens were sealed with acid resistant varnish leaving a 1mm window around the cervical margin interface. To detect marginal leakage, a 2% methylene blue buffered solution was used for 4 hours. The specimens were sectioned longitudinally and qualitatively evaluated by stereomicroscopy (45x), following a ranked score for the dentin cervical margin. The data were analyzed by Kruskal–Wallis test (a=0.05). Shear bond strength evaluation (SBS) - 80 bovine incisors were embedded and polished to obtain a flat standard surface on dentin. The surfaces were restored with Single Bond adhesive system and a composite resin subsequently inserted in a bipartite teflon matrix. The specimens were randomly divided into the four groups (n=20) described above for microleakage. Shear bond strengths were measured in a universal testing machine with a crosshead speed at 0.5mm/min. The data were analyzed by one way ANOVA test (a=0.05). No statistically significant influence of

thermocycling, mechanical load cycling, or the combination was observed for both microleakage and shear bond strength.

Introduction

A major goal of successful restorative treatment is the effective replacement of natural tooth structure. To effectively replace tooth structure, the restoration must be durable and functional. The durability of a restoration is largely based on the maintenance of the tooth/restoration interface. In order to help maintain the integrity of the restoration, the tooth/restoration interface must resist dimensional changes to prevent development of leakage and possible further deterioration of the restoration (Leibrock & others, 1999). In addition to durability and function, the esthetic aspect of a restoration is also a growing concern. The extensive use of composite resins and adhesive systems in anterior and posterior teeth has been made possible by the continued advancement of their mechanical and physical properties.

The control of polymerization shrinkage is an important step in achieving and maintaining the integrity of the restoration interface. Polymerization shrinkage resulting in stress at the dentin/restoration interface, allows for misadaptation of the material (Jorgensen & others, 1985), and consequently accelerates marginal leakage and possible deterioration of the restoration (Abdalla & Davidson, 1993; Alani & Toh, 1996; Dietschi & Herzfeld, 1998; Miyazaki & others, 1998). Even after controlling the effects of polymerization shrinkage, deterioration of the restoration could occur subsequently, due to chemical, thermal, and mechanical load stresses (Jorgensen & others, 1985; Abdalla & Davidson, 1993; Abdalla & Davidson, 1996; Mello & others, 1997).

The gold standard for evaluating the clinical potential of a restorative material is the controlled clinical trial. The constant and rapid evolution of adhesive materials increases the number of long-term clinical trials needed. However, due to increasing costs and the immediate demand for information, there is a need for surrogate methods. The establishment of an *in vitro* methodology capable of reproducing some *in vivo* challenges is crucial for better understanding of restorative materials behavior. The uses of mechanical and thermal cycling have been considered in laboratory studies of dental materials as potential methods to simulate *in vivo* challenges (Abdalla & Davidson, 1996; Mello & others, 1997; Leibrock & others, 1999).

The use of thermal cycling is frequently seen in laboratory studies evaluating microleakage (Darbyshire, Messer, Douglas, 1988; Yap, Stokes & Pearson, 1996; Alani & Toh, 1997; Yap, 1998; Hakimeh & others, 2000) and bond strength (Miyazaki & others, 1998; Leibrock & others, 1999). The effectiveness of this method in altering the restoration interface has been questioned by several groups (Alani & Toh, 1997; Yap, 1998; Hakimeh & others, 2000). It is difficult to compare these studies because the temperatures, number of cycles, and immersion times have not been standardized.

Several studies suggest that occlusal mechanical cycling could accelerate the deterioration of the dentin/restoration interface (DeLong & Douglas, 1983; Abdalla & Davidson, 1994; Abdalla & Davidson, 1996; Mello & others, 1997; Dietschi & Herzfeld, 1998). Some studies evaluating marginal leakage (Abdalla & Davidson, 1996; Yap, Stokes, Pearson, 1997; Munksgaard & Irie, 1998; Hakimeh & others, 2000) and bond strength (Williamson, Mitchell & Breeding, 1993; Leibrock & others, 1999) have included mechanical load cycling in their experimental protocol. Although, as with thermal cycling, it is difficult to compare the studies since they employed different load forces, number of cycles, and different cycle frequencies.

The aims of this study were to evaluate the influence of mechanical and thermal cycling on microleakage at the cervical margins of proximal slot restorations and shear bond strength on flat dentin surfaces. The null hypotheses tested were that there was no relationship between thermal and mechanical cycling to microleakage and shear bond strength.

Materials and Methods

I. Microleakage evaluation

Specimen preparation

One hundred and twenty fresh bovine incisors were cleaned of debris with curettes and pumice paste with a slow-speed handpiece and immersed in a 0.5% sodium azide saline solution. The incisal portion of the tooth was sectioned transversally 4 mm above the cemento-enamel junction (CEJ) at the mesial and distal surfaces allowing the configuration of a flat standard occlusal surface.

Proximal slot cavities on mesial (MO) surfaces were prepared with a #245 high-speed carbide bur (KG Sorensen Ltd. – Barueri, SP, Brazil 06454-920) under water spray. The cavity dimensions were 3 mm wide, 5 mm high (1mm below the CEJ) and 1.5 mm deep.

The cavities were restored with Single Bond adhesive system (3M/ESPE Dental Products, St. Paul, MN, USA 55144-100) following manufacturer's instructions: acid etched for 15 seconds, rinsed for 15 seconds, blotted dry, two consecutive coats of the adhesive were applied, lightly air dried, and light-cured for 10 seconds. The preparation was filled with a micro-hybrid composite resin Z-250 (3M/ESPE Dental Products) in two horizontal increments and light-cured for 40 seconds each, leaving a 1 mm overfill on the occlusal surface for mechanical load testing. During all restorative procedures the light intensity of the curing unit (Optilux/Demetron/Kerr Corp. - Orange, CA, USA 92867) was measured periodically by a radiometer (Demetron/Kerr Corp. Orange, CA, USA 92867) and was found to range from 520 to 560 mW/cm^2. After the restorative procedure, the specimens were stored in distilled water at 37°C for 24 hours. Afterwards, they were then finished and polished with Al_2O_3 abrasive discs (Sof-Lex Pop-on 3M/ESPE Dental Products). The teeth were then randomly divided into 4 groups (n=30):

G1= Control group (no thermal and mechanical load cycling)

G2= Thermal cycling (2,000 cycles; 5 – 55 °C)

G3= Mechanical load cycling (50,000 cycles; 80N)

G4= Thermal cycling (2,000cycles; 5 – 55 °C) and mechanical cycling (50,000 cycles; 80N)

Thermal cycling and mechanical load cycling procedure

The specimens from groups 2 and 4 were subjected to 2,000 cycles in a thermocycling machine (MCT2 - AMM - 2 INSTRUMENTAL, Sao Paulo, SP, Brazil 04282) with two baths at 5 ± 2°C and 55 ± 2°C with a dwell time of 60 seconds and a transfer time of 7 seconds between each bath.

Specimens from groups 3 and 4 were submitted to mechanical load cycling. The specimens had part of their root embedded in epoxide resin (Buehler Ltd. Lake Bluff, IL, USA 60044) in order to obtain a flat occlusal surface perpendicular to the long axis of the tooth. The cyclic mechanical loading device consisted of 4 stainless steel pistons in which a polyacetal cylinder tip was attached to the end. The polyacetal tips were placed in contact with the restoration (Figure 1). The loading device delivered an intermittent axial force of 80 N at 3 cycles/sec totaling 50,000 cycles.

Immersion in dye solution and microleakage evaluation

After thermal and mechanical load cycling, the apices and the occlusal portion were filled with epoxy resin (Araldite Ciba – São Paulo, SP, Brazil 04706-900) to avoid infiltration of the dye solution. The entire surface of each tooth was then coated with 2 layers of acid resistant varnish, except for a 1 mm width around the cervical margin. The teeth were immersed in a 2% methylene blue buffered solution for 4 hours; they were then thoroughly rinsed under tap water for 10 minutes.

The teeth were hemi-sectioned longitudinally, in a mesio-distal direction through the center of the restoration with a double-faced diamond disc (KG Sorensen Ind. e Com. Ltda.) mounted on a straight low speed handpiece with water spray. For each restoration, two sections were obtained; the section with the highest score of leakage was considered for microleakage analysis since it represents the most severe dye penetration of the restoration. The sections were evaluated by three independent calibrated examiners on a stereomicroscope (MEIJI-EMZ-TR/Meiji Techno Ltd. San Jose, CA, USA 95131) at 45x magnification. The following criteria were used to score the extent of leakage at the cervical margin (Figure 2):

0 = No dye penetration;

1 = Dye penetration up to 1/3 of the cavity depth;

2 = Dye penetration up to 2/3 of the cavity depth;

3 = Dye penetration up to the entire cavity depth;

4 = Dye penetration into axial wall of cavity.

II. Shear bond strength test

Specimen preparation

Eighty extracted bovine incisors were collected, cleaned and stored in a 0.5% sodium azide saline solution. In each tooth, a piece from the coronal facial surface was sectioned with a double face diamond disk (KG Sorensen - Barueí, SP, Brazil 06454-920) and mounted in a 3/4 inch diameter PVC ring, parallel to the base of the ring. The rings were then filled with self-curing epoxide resin (Buehler Ltd.) to set the teeth. The embedded teeth were ground on a water-cooled mechanical grinder (Maxigrind–Solotest - São Paulo, SP, Brazil 01328) using 320 grit Al_2O_3 abrasive paper (Carborundum Abrasivos – Ribeirao Preto, SP, Brazil 30180-070) to expose the dentin, and polished with 400 and 600 grit Al_2O_3 abrasive paper to obtain a 5-6 mm area of flat standardized dentin surface. They were then stored in distilled and deionized water at 37°C for 24 hours.

Before the surface treatment, a 3 mm circular area was left uncovered as a bonding site by placing a piece of vinyl tape with a 3 mm diameter punched hole over the dentin. Then the Single Bond adhesive system was applied following manufacturer's instructions.

A 3 mm diameter and 3 mm high bipartite teflön ring mold was clamped to the dentin surfaces such that the mold was positioned over the treated dentin. The mold was bulk filled (Z-250) and light-cured (Optilux/Demetron) for 40 seconds, and then light-cured again for an additional 40 seconds after removing the mold. The light intensity was measured periodically by a radiometer. It ranged from 520 to 560 mW/cm^2. The specimens were immersed in distilled and deionized water at 37°C for 24 hours.

Then the teeth were randomly divided into 4 groups (n=20):

G1= Control group (no thermal and mechanical load cycling)

G2= Thermal cycling (2,000 cycles)

G3= Mechanical load cycling (50,000 cycles)

G4= Thermal cycling (2,000 cycles) and mechanical cycling (50,000 cycles)

Thermal and mechanical load cycling procedure

Thermal and mechanical load cycling procedures were performed as previously mentioned. The specimens from groups 3 and 4 were submitted to mechanical load cycling with the polyacetal tips placed in contact with the top of the composite resin cylinder. (Figure 1)

Bonding test

Each specimen was mounted in a custom apparatus attached to a universal testing machine (EMIC Ltd São José dos Pinhais, PR, Brazil 83020-250) with the dentin surface parallel to the machine's trajectory. A compressive load was applied using a steel knife edge placed over the specimens so that the shear force was applied directly to the interface. The specimens were loaded to fail at a crosshead speed of 0.5mm/minute. The values were recorded and the shear bond strengths were calculated for each sample. Means and standard deviations were expressed in MPa. For fracture mode analysis, specimens were evaluated under a stereomicroscope (MEIJI-EMZ-TR/Meiji Techno Ltd.) at 20x magnification and classified as adhesive, cohesive (in dentin and composite), or mixed failures (adhesive and cohesive).

Statistical analysis

For microleakage evaluation, the scores of the 3 examiners were analyzed using the reproducibility Kappa test between examiners. To determine significant differences between groups, the median scores obtained by the 3 examiners were analyzed using the Kruskall-Wallis non-parametric analysis of variance test (a=0.05). Shear bond strength data were analyzed using the parametric analysis of variance test (one-way ANOVA - a=0.05).

Results

I. Microleakage analysis

The Kappa Test showed 0.79 agreement between examiners 1 and 2, 0.95 between examiners 1 and 3, and 0.74 between examiners 2 and 3. The mean agreement was 0.83, indicating a good level of reliability for the scores evaluated. The

Kruskal-Wallis non-parametric test indicated no statistically significant differences between the control group (G1) and the experimental groups – G2, G3, G4 ($p=0.52$). Thermal and mechanical load cycling did not have any effect on the microleakage values when compared to the control group. The mean rank of the dye penetration values are listed in Table 1 and the frequency of microleakage scores are described in Table 2. The most common score was 1(Figure 3), demonstrating a low extent of dye penetration and satisfactory sealing ability of the groups evaluated.

II. Shear bond strength analysis

No statistically significant differences ($p=0.49$) were found for the bond strength values between the 4 groups evaluated. Table 1 describes the mean and standard deviations obtained for all groups. The use of thermal and mechanical load cycling did not affect the bond strength values of the experimental groups (G2, G3, G4) when compared to the control group (G1). For the fracture mode analysis, adhesive failure was most commonly observed. All groups presented with more than 95% of adhesive failure and no differences were observed among the groups. Mixed fracture was observed for all other specimens (5%) and no cohesive failures were observed.

Discussion

The unpredictability of the dentin substrate is a serious problem when bonding restorative materials (Pashley & others, 1993). For the microleakage evaluation, the cervical margins of the restorations were positioned 1 mm below the CEJ, due to the critical adhesion in this area (Cagidiaco & others, 1997). The tubules in the cervical region are parallel to the cavity preparation, which impedes the formation of conventional tags. In addition, more intertubular dentin and less dentin tubules are exposed, increasing the area of hybrid layer formation and resulting in higher bond strength values when compared to surfaces in which tubules are perpendicular to the bonded interface (Ogata & others, 2001).

Shear bond strength specimens were prepared on flat superficial dentin from the facial surface. The shear bond strength test has been criticized due to the occurrence of cohesive failures with current adhesive systems. This test reproduces a compressive stress situation present in restorations. In addition, it has been shown to be a reliable test and is currently used for bond strength evaluation in different substrates and conditions (Cardoso, Braga & Carrilho, 1998; Miyazaki & others, 1998; Leibrock & others, 1999)

In this study, bovine teeth were used in place of human teeth. Previous studies (Nakamichi, Iwaku & Fusayama, 1983; Reeves & others, 1995) have shown the similarity of bovine and human teeth in bonding tests. The major advantage of employing bovine teeth is the possibility of controlling for the age of the substrate (2 years).

The application of thermal cycling and mechanical load cycling did not influence the values of microleakage and shear bond strength in the present study. The use of thermocycling has been thoroughly investigated (Prati & others, 1994; Miyazaki & others, 1998; Hakimeh & others, 2000; Yoshida, Kamada & Atsuta, 2001). Variation can be observed depending on dye stains, number of cycles, temperatures, and materials employed. The most common dye penetration stains employed are silver nitrate and methylene blue; both considered acceptable markers for microleakage evaluation (Alani & Toh, 1997). The quantity of cycles and the temperatures used seem to be the major difference among different studies (Prati & others, 1994; Yap, Stokes & Pearson, 1996; Cardoso & others, 1999; Hakimeh & others, 2000). In the present study, 2,000 cycles were used as an average of recent articles. The use of intermediary baths and different temperatures has been described, but the use of ISO standardization (5-55°C) allows for a better comparison between studies.

Controversial results have been reported for the influence of thermal cycling on microleakage. Darbyshire, Messer & Douglas, 1988; Prati & others, 1994; Chan & Gly-Jones, 1994 reported no effect of thermal cycling on microleakage. Recently, Hakimeh & others, 2000 reported increased microleakage in class V restorations after 2,880 cycles (4-60°C). Several differences in the methodology of all these studies can be seen such as number of cycles, temperature, and number of baths. In addition, the type of restorative material (amalgam, glass ionomer cements, composite) used and the preparation (Class I, II or V) also varied. It is difficult to compare the data of the present study with those of other studies due to differences in methodology; however number of cycles seems to be of major importance since different values may result, according to the number of cycles used, independent of the material and force employed. Further studies should be developed to evaluate different number of cycles and different materials.

The simulation of thermal stress for shear bond strength evaluation has been reported by some groups. Miyazaki & others, 1998 and Yoshida & Atsuta, 1999 reported no influence of thermal stress when using 10,000 and 20,000 cycles respectively. Differences in bond strength, when subjected to thermocycling, are more closely related with adhesion between material/material, than to material/substrate (dentin,enamel). Yoshida & others, 2001 reported a decrease in bond strength between porcelain and resin after 50,000 thermal cycles. Leibrock & others, 1999 evaluating adhesion of porcelain repair systems, observed changes in shear bond strength with 2,400 thermal cycles and 480,000 mechanical cycles depending on the materials employed. Miyazaki & others, 1998 when comparing different numbers of thermal cycles, observed a decrease in bond strength at 30,000 cycles for some adhesive systems. On the other hand, an increase in shear bond strength was observed after thermal cycling in enamel/material (Hosoya, 1994) and material/material (Atta, Smith & Brown, 1990; Yoshida, Kamada & Atsuta, 2001). Considering the data of all studies cited and the current study, we suggest that the influence of thermocycling is dependent on the number of cycles, restorative materials, and dental substrates. In the present study thermal cycling was unable to affect the bonding most likely due to both an insufficient number of cycles and high bond strength values.

The use of mechanical load cycling has been studied due to the potential of simulating mastication. In microleakage evaluations, load is applied with different tips, loading sets, number of cycles, and load forces. In the present study, the use of a cylindrical polyacetal tip touching only the material, aimed to fatigue the restoration, was used. The force of 80 N was chosen as an average of the masticatory forces observed by Anderson, 1956. It is difficult, if not impossible, to simulate the occlusal forces, due to the variation of age, sex, type of tooth, and food. These factors interfere with the force employed; however, the simulation of a mean force is necessary for further comparison between studies.

Abdalla & Davidson, 1996; Mello & others, 1997; Ausiello & others, 1999 using spherical tips touching only cusps, observed differences in microleakage values when applying 4000 cycles at 125 N. In these studies the dye immersion was performed during loading, as opposed to our study. According to Jorgensen & others, 1976 during loading, temporary gaps are formed allowing for microleakage detection. However, other studies have shown results similar to our study when evaluating the effect of loading on microleakage. Prati & others, 1994 using a spherical tip touching only an MOD restoration did not observe any changes in microleakage using 17 Kgf for 1,440 cycles. In another study (Hakimeh & others, 2000), no effect on leakage was seen using 50,000 mechanical loading cycles (100N) on class V restorations. We suggest, according to the information obtained from the studies cited and the current study, that the restorative material, tip shape, location of force application, number of cycles, and load force are responsible for the varying results.

The use of load cycling to analyze shear bond strength was studied by Leibrock & others, 1999 and Williamson, Mitchell & Breeding, 1993. Both studies evaluated the adhesion between material/material. For the present study, a previous pilot study was conducted in order to evaluate the capacity of the specimen to support 80N for 50,000 cycles as proposed for the previous microleakage evaluation. The shape of the cylindrical resin cone (3 mm high and 3 mm in diameter) allowed the use of the load and no specimen was lost during the mechanical loading. In our current study, bond strength was not influenced by mechanical loading. The current adhesive systems on the market have been shown to produce high bond strength values and good sealing ability (Cardoso, Braga & Carrilho, 1998; Cardoso & others, 1999; Ogata & others, 2001). The ethanol-based adhesive Single Bond, employed in the present study, is a representative one bottle system that performs well in most studies. We suggest that the high quality of adhesion achieved by recent adhesive systems might limit the possible effect of mechanical cycling on bond strength and microleakage.

Further studies using different numbers of cycles and load will be conducted in order to observe a possible influence of increased numbers of cycles on bond strength. In addition, the evaluation of microtensile and nanoleakage will also be performed in order to evaluate the influence of thermal and mechanical load cycling.

Conclusion

According to the results the null hypothesis was accepted. Thermocycling and mechanical loading showed no effect on microleakage and shear bond strength either independently or in combination.

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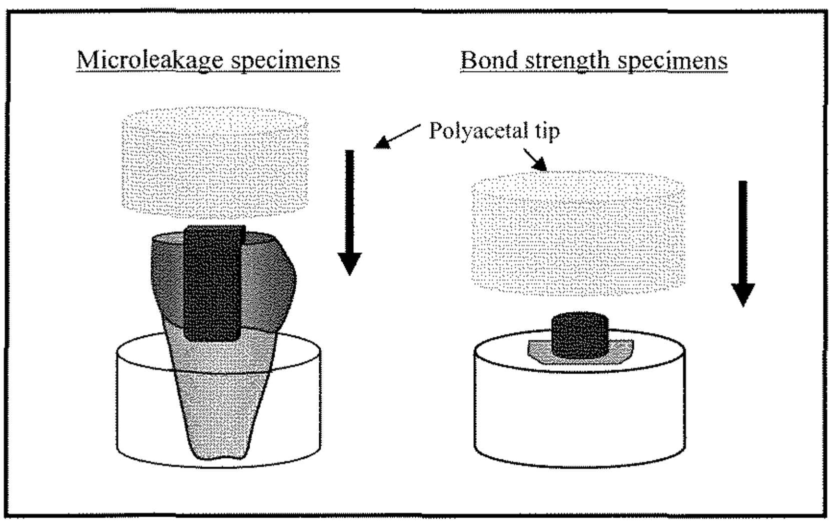

Figure 1 - Illustration of the specimen shapes and incidence of the polyacetal tip for mechanical loading cycling. The load was applied only to composite resin. Black arrows show the axial direction of the force.

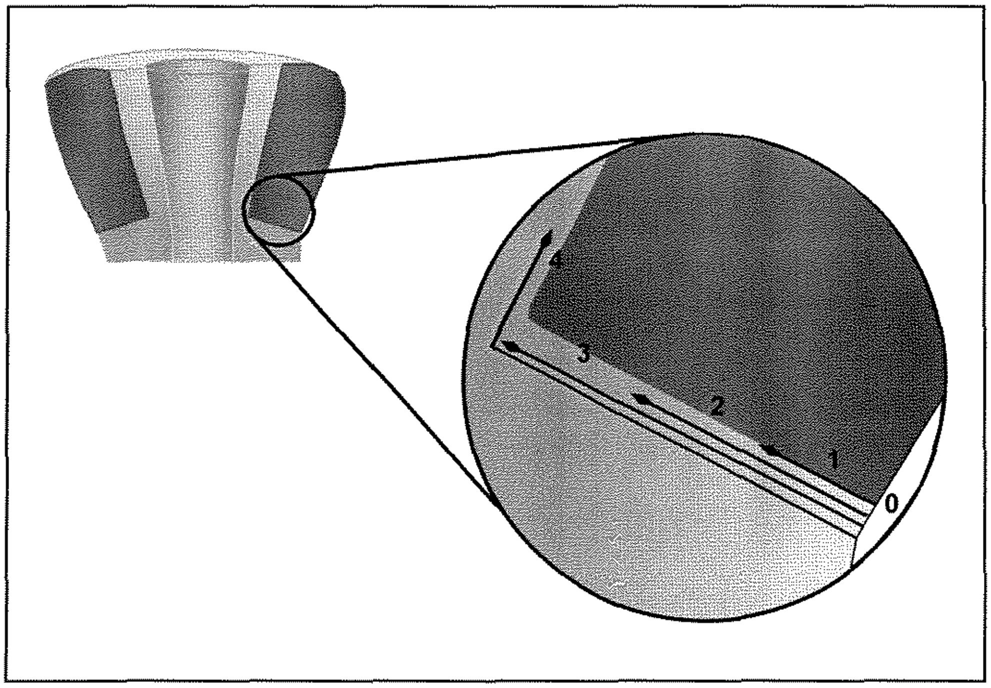

Figure 2 - Schematization of the criteria employed to analyze the extent of leakage on cervical margin (dentin).

Table 1 - Means and standard deviation for shear bond strength values and median and mean ranks of microleakage values.

	Results			
Groups	Shear bond strengths values n=20		Microleakage values n=30	
	mean	SD	median	mean ranks
G1 - Control	20.76	3.58	1	45.5
G2 - Thermal cycling	19.61	3.79	1	40.5
G3 - Mechanical cycling	21.13	3.60	1	54.6
G4 - Thermal cycling/mechanical cycling	21.02	3.54	1	47.4

Table 2 - Frequency of microleakage scores obtained for each experimental group.

Groups	Dye penetration scores					total
	0	*1*	*2*	*3*	*4*	
G1	2	16	4	6	2	30
G2	3	14	3	8	2	30
G3	2	19	2	2	5	30
G4	5	17	3	2	3	30

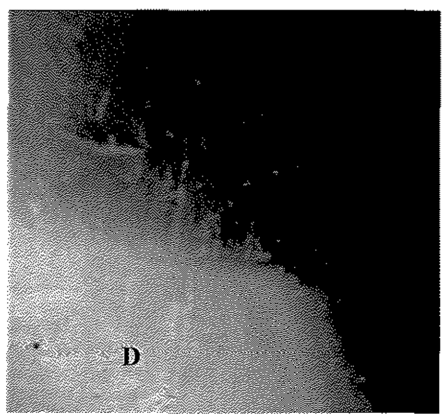

Figure 3 – Photograph of microleakage with dye penetration score 1 between dentin (D) and composite (C).

CAPÍTULO 2

Influence of thermal and mechanical load cycling on dentin microtensile bond strength

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Short title: Influence of thermal and mechanical load cycling on bond strength

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Clinical significance: *The combination of thermal and mechanical cycling adversely affected bond strengths, resulting in a possible simulation of the oral stresses incurred during the lifespan of a restoration.*

Summary

To evaluate the effect of thermal and mechanical cycles on dentin bond strength to cervical margins of class II restorations. Eighty box-type class II cavities were prepared on surfaces of bovine incisors. The cavities were restored with Single Bond (3M-ESPE) and Z-250 composite (3M-ESPE) according to manufacturer's instructions. The teeth were divided into 4 groups: G1- Control, G2- Thermal cycling (2,000 cycles, 5-55°C), G3- Mechanical cycling (100,000 cycles; 50N), G4- Thermal and mechanical cycling (2,000 cycles 5-55°C/ 100,000 cycles; 50N). The restorations were sectioned perpendicular to the cervical bonded interface into 0.7 ± 0.2 mm thick slabs. The slabs were further trimmed at the interface to 1.4 ± 0.2 mm with a fine diamond bur to produce a cross-sectional surface area of ca.1mm^2. All specimens were then subjected to microtensile bond testing. Means and standard deviations were expressed in MPa. The bond strength data were analyzed by one way ANOVA and Fisher's PLSD test ($p<0.05$). Fracture mode analysis was performed using SEM. Bond strengths were significantly lower when thermal and mechanical cycling were performed [G4- 22.41 (8.57)] when compared to the other groups [G1- 28.15 (14.03); G2- 27.60 (10.14); G3- 27.59 (8.67)]. No differences were observed between groups 1, 2, and 3. Interfacial fracture of the control (G1) and thermocycling (G2) groups mainly occurred

between the deepest portion of the adhesive resin and the top layer of the demineralized dentin (Interphase). Mixed failure was predominant and increased when mechanical cycling was applied (G3 and G4).

Introduction

The control of composite polymerization shrinkage is the first step in achieving and maintaining the integrity of the restoration interface. Polymerization shrinkage results in stress at the dentin/restoration interface, allows for non-adaptation of the material (Jorgensen & others, 1985), and consequently accelerates marginal leakage and deterioration of the restoration (Abdalla & Davidson, 1993; Alani & Toh, 1997; Miyazaki & others, 1998). Deterioration of the restoration could occur subsequently, due to chemical, thermal, and mechanical load stresses (Jorgensen & others, 1985; Abdalla & Davidson, 1993; Abdalla & Davidson, 1996; Mello & others, 1997).

The establishment of *in vitro* methodologies using different types of stresses present in the oral environment is important, since the constant and rapid evolution of adhesive materials does not allow for long-term clinical trials. The use of thermal and mechanical stresses is used in *in vitro* studies in order to mimic the natural aging process of the restoration. Studies evaluating microleakage have shown conflicting results regarding the effectiveness of both mechanical (Darbyshire, Messer & Douglas, 1988; Abdalla & Davidson, 1993; Prati & others, 1994; Abdalla & Davidson, 1996; Hakimeh & others, 2000; Nara &

others, 2002) and thermal stresses (Darbyshire, Messer & Douglas, 1988; Chan & Glyn-Jones, 1994; Hakimeh & others, 2000;), and very limited bond strength studies have been conducted associating these stresses (Miyazaki & others, 1998; Ausiello & others, 1999; Nikaido & others, 2002a; Nikaido & others, 2002b).

With the recently developed microtensile bonding test (Sano & others, 1994), measurement of bond strength to small surface areas (0.5-1.0mm^2) have become possible. It permits the testing of irregular surfaces and cavities, which are more clinically relevant than flat surfaces, but was still not possible with conventional shear and tensile bond tests. The benefit of having the possibility of evaluating small areas such as cervical, gingival, and axial walls as well as the pulp floor of Class I, II, V preparations is enormous and closer to the clinical situation since clinicians usually do not bond to flat surfaces with a very small C-factor. Studies have shown (Nikaido & others, 2002b) a negative influence on bond strength when tested relative to different cavities walls.

The aim of this study was to evaluate the effect of mechanical and thermal cycling on microtensile bond strength (μTBS) and failure mode pattern at the cervical margins of class II slot restorations. The null hypotheses tested was that thermal and mechanical cycling would not affect bond strength and failure modes.

Methods and Materials

Eighty bovine incisors were selected, cleaned of debris with curettes and pumice paste in slow-speed, and stored in a 0.1% sodium azide saline solution. Occlusal surfaces were cut at the level of marginal ridges under water refrigeration, preparing a flat surface 4 mm above the cementum-enamel junction.

Class II slot preparations were prepared on the mesial surface following the dimensions: 3 mm of width, 5 mm of height (starting at the marginal ridge and with gingival margins in dentin) and 1.5 mm of depth towards the pulp chamber. All cavities were prepared using carbide burs (#245 KG Sorensen, Barueri, SP, Brazil 06454-920) mounted in a high-speed handpiece under water refrigeration. The burs were replaced after every 5 preparations.

Cavities were restored with Single Bond adhesive system (3M-ESPE Dental Products, St. Paul, MN, USA 55144-100) and composite resin Z-250 (3M-ESPE Dental Products). The adhesive system was applied according manufacturers' instructions: acid etch for 15 seconds, rinse for 15 seconds, blot dry, apply two consecutive coats of the adhesive, lightly air dry, and light-cure for 10 seconds. The preparation was filled with a micro-hybrid composite resin Z-250 (3M-ESPE Dental Products) in two horizontal increments and light-cured for 40 seconds each. A 1 mm overfill was left on the occlusal surface to enable mechanical load cycling on the restoration only. During all restorative procedures the light intensity (Optilux 501/Demetron/Kerr Corp. - Orange, CA, USA 92867) was measured periodically by a radiometer (Demetron/Kerr Corp.) and ranged from 520 to 560 mW/cm^2. After the restorative procedure, the specimens were stored in distilled/deionized water at 37°C for 24 hours. Afterwards, they were finished and polished with Al_2O_3 abrasive discs (Sof-Lex Pop-on/3M ESPE). The teeth were then randomly divided into 4 groups:

G1= control group (no thermal and mechanical load cycling)

G2= thermal cycling only (2,000 cycles, 5-55 °C)

G3= mechanical load cycling only (100,000 cycles / load = 50N)

G4=thermal cycling (2,000 cycles, 5-55 °C) and mechanical cycling (100,000 cycles / load = 50N)

The specimens subjected to mechanical load cycling had part of their roots embedded in epoxy resin (Buehler Ltd. Lake Bluff, IL, USA 60044) in order to obtain a flat occlusal surface perpendicular to the long axis of the tooth.

Thermal cycling and mechanical load cycling procedure

Specimens from groups G2 and G4 were subjected to 2,000 cycles in a thermocycling apparatus (MCT2 - AMM - 2 INSTRUMENTAL, Sao Paulo, SP, Brazil 04282) with two baths at 5 ± 2°C and 55 ± 2°C each with a dwell time of 60 seconds and a transfer time of 7 seconds between each bath.

Specimens from groups G3 and G4 were subjected to mechanical load cycling. The cyclic mechanical loading device used was a Leinfelder Wear Test Apparatus (custom by Dentsply/Caulk Technical Research, Milford, DE, USA 19963-0359), modified for loading test. The apparatus consisted of 4 stainless pistons in which a polyacetal cylindrical tip was attached to the end. The polyacetal tips were placed in contact with the restoration. The loading device delivered an intermittent axial force of 50 N at 3 cycles/sec totaling 100,000 cycles. (Figure 1-A) For group G4, thermal cycling was performed first and the mechanical cycling was performed after.

Microtensile Bonding Test

After thermal and mechanical load cycling, a 3 mm-thick resin block was built on the outermost surface of the restoration in order to produce grips for the microtensile bond test. The restorations were sectioned perpendicular to the cervical bonded interface of each tooth into 0.7 ± 0.2 mm thick slabs (n = 2 per restoration) with a slow speed diamond wafering blade (Buehler-Series 15LC Diamond) and constant water coolant (Figure 1-B). The composite/dentin interface was further trimmed at the interface to 1.4 ± 0.2 mm with a fine diamond bur to produce a cross-sectional surface area of ca.1mm^2 (Figure 1-B). All specimens were then glued with Zapit (DVA, Corona, CA, USA, 91720) to a Ciucchi's jig which was mounted on an universal testing machine (EZ Test, Shimazu Co., Kyoto, Japan 101-8448) and subjected to microtensile bond testing at a cross-head speed of 1 mm/min. Means and standard deviations were calculated and expressed in MPa. Statistical analysis was performed using ANOVA and Fisher's PLSD test ($p < 0.05$).

Fracture mode analysis

For fracture mode analysis, specimens were stored in 10% neutral buffered formalin solution after debonding for at least 8 h. All specimens were then mounted on stubs, gold sputter coated (Polaron E-5200 energy Beam Sciences, Agawan, MA, USA 01001-2925), and examined in a scanning electron microscope (model 6500, JEOL Corp., Peabody, MA, USA 01960). Fracture modes were classified as: failure between the deepest portion of the adhesive resin and the top layer of the demineralized dentin [Interphase] (Nakabayashi & Pashley, 1998), cohesive failure in composite, cohesive failure in adhesive, and mixed failure (association of two or more failures). Fractures were calculated according to the percentage present in each specimen.

Results

1. Microtensile bond strengths

Statistically significant differences were observed between groups ($p < 0.05$) and are described in Table 1. Bond strengths were significantly lower when thermal and mechanical cycling were performed to the same specimens (G4- 22.4 ± 8.6 MPa) as when compared to the other groups (G1- 28.2 ± 14.0 MPa; G2- 27.6 ± 10.1 MPa; G3- 27.6 ± 8.7 MPa). No differences were observed between the control, thermal cycling only, and mechanical cycling only groups.

2. Fracture mode analysis

The fracture mode analysis results are shown in Figure 2. All groups presented the 4 types of failure modes [interphase (Figure 3A), cohesive in adhesive (Figure 3B) or composite (Figure 3C), and mixed]. Failure at the interphase was observed mostly for the control group (G1) (Figure 4A and B) and thermal cycling group (G2). Adhesive failure was only observed for control group specimens in a very low percentage rate. Cohesive failure in composite was observed in all groups especially G2 (thermal cycling). Mixed failure was predominant and increased when mechanical cycling was applied (G3 and G4). Fracture at the interphase showing the tags in parallel directions (Figure 5A) was also observed especially in mechanical cycling groups (G3 and G4), and indicates a failure in the hybrid layer. The presence of water voids within the interphase was observed in several specimens (Figure 5B) and could have some effect on the bond strength values.

Discussion

The goal in adhesive dentistry is to develop materials which are able to replace and act as the biological hard dental structure. Sealing ability (nanoleakage and microleakage) and bond strength (tensile and shear bond strength) are the two main types of laboratory tests used to evaluate the performance of restorative systems. The feasibility of using these tests to evaluate restorations placed in cavity preparations permits the evaluation of a specific material in a condition that is clinically relevant. With the introduction of microtensile bond testing, it became possible to test clinically relevant substrates such as class I, II, and V preparations, caries-affected dentin and sclerotic dentin. The advantage of the microtensile bond test is to allow evaluation of small areas and different substrates (Sano & others, 1994; Pashley & others, 1999), which is not possible using conventional shear and tensile tests.

It has been reported that tensile bond strengths were lower at the apical wall than at the occlusal walls of cervical wedge shaped cavities (Ogata & others, 1999). Most of these differences can be explained due to the direction of the tubules (Ogata & others, 2001). They demonstrate that for tubules oriented parallel to the bonded interface, higher values were observed when compared to tubules oriented perpendicular to the interface. A thicker hybrid layer was observed for the group with parallel dentin tubule orientation. In the present study, dentin tubules were oriented parallel to the cervical wall, which allows for acceptable values of bond strength but still lower when compared to flat surface. One explanation for the results may be the effect of cavity configuration factor (C-factor) on bond strength data. C-factor is the ratio of the bonded surface area to the unbounded or free surface area (Feilzer, de Gee & Davidson, 1987). Bouillaguet & others (2001) reported a 20% reduction in bond strength of class II cavities walls compared to flat dentin surface.

In the fracture mode analysis, the presence of water voids at the fracture interface was used (Figure 5B). During the treatment of the cavity, due to its configuration, it may have been difficult to control the blot drying and application of the adhesive system. The influence of adhesive layer thickness was studied by Zheng & others (2001). In their study, the use of an ethanol/water-based adhesive system (Single Bond) resulted in a decrease of bond strength values for thicker adhesive layers. According to the authors, in thicker layers the solvent may not evaporate, leading to poor polymerization, and a subsequent decrease in bond strength. Also the use of a moist bonding technique at cavities makes blot drying a critical step, and the presence of excess water may turn difficult the evaporation of the solvent. According to our data, it is difficult to know if the incomplete evaporation of the solvent or the presence of excess water in the demineralized dentin, or the association of both were responsible for the voids present at the interface.

In this study, bovine teeth were used in place of human teeth. Previous studies (Nakamichi, Iwaka & Fusayama, 1983; Reeves & others, 1995) have shown the equivalence of bovine and human teeth in bonding tests. The major advantage of using bovine teeth is the ability to control age, sclerosis, and amount of wear of the substrate (average age of 2 years).

The use of thermocycling in *in vitro* mechanical tests evaluation has been thoroughly investigated (Prati & others, 1994; Miyazaki & others, 1998; Hakimeh & others, 2000). The quantity of cycles and the temperatures used seem to be the major difference among different studies (Prati & others, 1994; Alani & Toh, 1997; Hakimeh & others, 2000). Controversial results have been reported for the influence of thermal cycling on microleakage. Darbyshire, Messer & Douglas (1988), Prati & others (1994), and Chan & Gly Jones (1994) reported no effect of thermocycling on microleakage. Recently, Hakimeh & others (2000) reported increased microleakage in class V restorations after 2880 cycles (4-60°C).

Some studies report no influence of thermal stress when using a low number of cycles for shear bond strength (Miyazaki & others, 1998; Yoshida & Astuta, 1999). Nikaido & others (2002b), observed no influence of 2000 thermal cycles (5-55 °C) on μTBS of flat dentin surface. The same authors observed a decrease in bond strength when thermocycling was performed in class I preparation. But the authors used concomitant load cycling; therefore it is difficult to evaluate the real influence of thermal cycling only on the outcome of the data. In the present study, the influence of thermal cycling alone was not observed, but the use of thermal cycling and mechanical cycling in the same specimens reduced bond strength significantly.

The use of mechanical load cycling has been studied due to the potential capability of simulating mastication. In the present study, the use of a cylindrical polyacetal tip touching only the material, aimed to fatigue the restoration, was used. A force of 50 N was chosen in order to simulate an average of constant load found during mastication (Anderson, 1956).

For microleakage evaluation using spherical tips touching cusps, Abdalla & Davidson (1996); Mello & others (1997); Ausiello & others (1999), observed differences when applying 4000 cycles at 125 N. Using a spherical tip touching only an MOD restoration, Prati & others (1994) did not observe any changes in microleakage using 17 Kgf for 1,440 cycles. In another study (Hakimeh & others, 2000), no effect of mechanical loading was seen using 50,000 mechanical loading cycles (100N) on class V restorations. Nara & others (2002) observed that the association of thermal and load cycling was effective to examine microleakage of cervical composite restorations. This data is in accordance to our findings, where the use of thermal and mechanical load cycling in the same specimens resulted in a negative effect at the bonded interface, reducing the bond strength values.

The evaluation of mechanical load cycling on μTBS from cavity pulp floors was recently documented (Nikaido & others, 2002a, Nikaido & others, 2002b). Nikaido & others (2002a) evaluated the influence of 50,000 cycles on class I preparations restored with self etching or total etch adhesive systems. However, they did not report the influence of mechanical load cycling on μTBS. In accordance to our study, the influence of mechanical cycling alone was not observed in the present study. In another study, Nikaido & others (2002b) using the same load force (50N) and number of cycles (100,000) on class I restorations employed in the present study, and associated with concomitant thermocycling, related a decrease in μTBS of a self etching adhesive system. But according to their study, it seems doubtful that thermal stress would reach the dentin-adhesive interface on the pulp floor of a class I restoration. According to the present study, only the use of concomitant thermal cycling with mechanical load cycling could influence the results.

The use of different adhesive systems and cavity shapes may be reasons for the different results found. Adhesive systems have been shown to behave differently according to the substrate to which they are bonded (Yoshikawa & others, 1999) and to operator variation (Finger & Balkenhol, 1999). The cavity shape used in the present study permitted the evaluation of an interface where dentin tubules were parallel. This may have been the reason for the higher bond strength values compared to tubules directed perpendicular (pulp floor) to the interface as reported by Nikaido & others (2002a). In Class II cavities, the restoration is exposed to a lower C-factor than for Class I, which could be associated with the better bond strength values for the Class II.

According to the fracture mode analysis, the use of thermal cycling increased the cohesive failure of resin, reduced the mixed failures, and presented similar interface failure when compared to the control group. The results of the current study suggest that thermal cycling promoted a slight change in fracture mode, but not enough to influence the μTBS values (Group 2). When mechanical load cycling was performed alone, no interfacial failure was observed and mixed failure increased. The same behavior was observed when thermal and mechanical load cycling were performed on the same specimens (Group 4), resulting in more than 90% mixed failures. It can be speculated that for this group, because thermal cycling was performed first, it promoted stress on the interface. Then, mechanical cycling was applied to these specimens that had already been exposed to a modified environment due to thermocycling.

Consequently, the effect of loading was accelerated by the thermocycling, resulting in significantly lower bond strength. The mixed fracture mode consisted of the 3 types of fracture, weather the three appeared together or not. It is difficult to specify the localized stress point at the interface. Observing that fracture at the interphase was reduced with load-cycling, it can be suggested that the mechanical loading could weaken the adhesive resin at the bonded interface. Weakening of the adhesive resin due to mechanical loading is a very important issue in restorative dentistry, since the demand of posterior composites by patients has significantly increased. The recent adhesive technologies that combine the primer and adhesive may be at a greater risk for failure if the solvent is not adequately removed by air drying. Although technique sensitivity and type of adhesive resin may vary, future studies should focus on determining the durability of adhesive resins under mechanical loading.

Conclusion

The null hypothesis that the thermal cycling and mechanical load cycling would not influence bond strength was rejected when both were used in the same specimens, reducing the bond strength.

Thermal and mechanical cycling combined, adversely affected bond strengths and failure modes. Simulation of the oral condition might be crucial to better evaluate and understand the performance of adhesive materials.

Acknowledgment

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Figure 1. A- Illustration of the load application on the occlusal surface. B- Illustration of the restoration section and slab configuration for microtensile bond strength test.

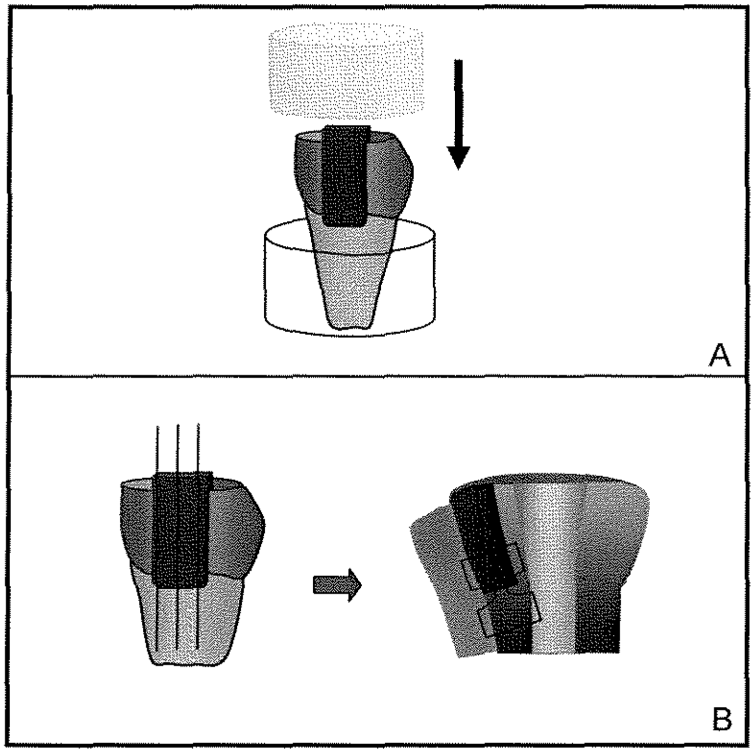

Table1. Means and standard deviation for microtensile bond strength values.

	Thermal Cycling (2,000 cycles 5-55°C)	*Mechanical Cycling (100,000 cycles - 50N)*	*Mean (SD) MPa*	*$p<0.05$*
G1	-	-	28.15 (14.03)	a
G2	+	-	27.60 (10.14)	a
G3	-	+	27.59 (8.67)	a
G4	+	+	22.41 (8.57)	b

(+ = performed; - = not performed)

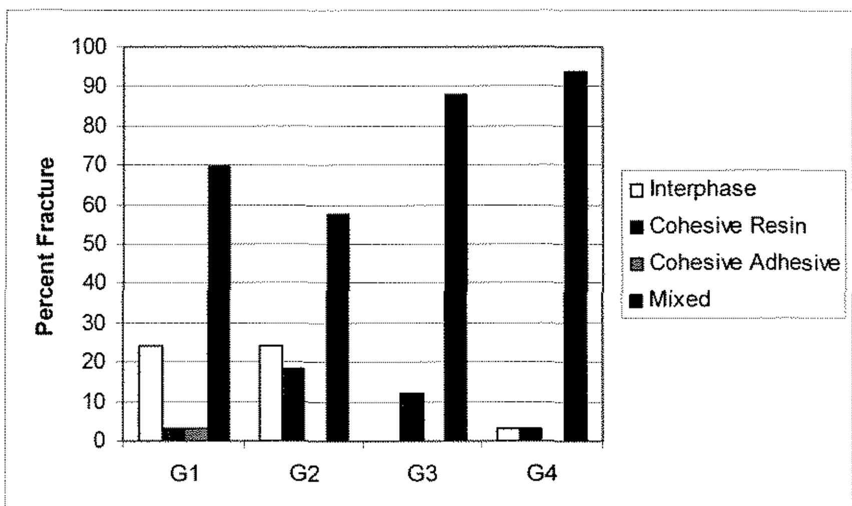

Figure 2. Fracture mode analysis of the groups evaluated. G1- control, G2- Thermal cycling, G3- Mechanical load cycling, G4- Thermal and Mechanical load cycling.

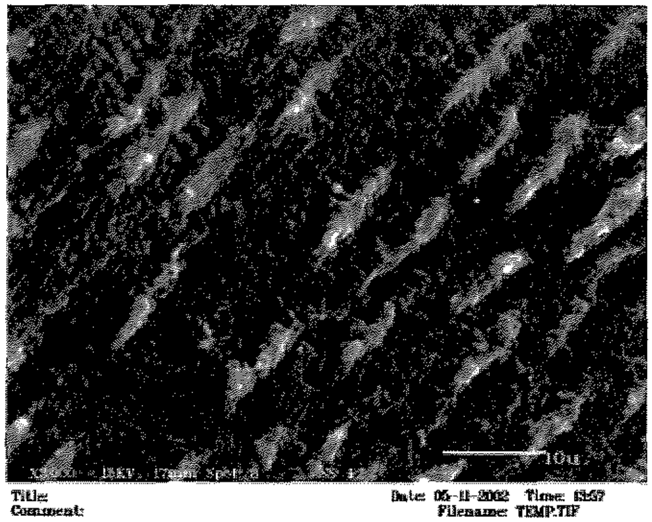

Figure 3. A- SEM micrograph of an interphase failure. Parallel direction of dentin tubules to the cervical wall are identified by presence of tags underneath the face of fracture.

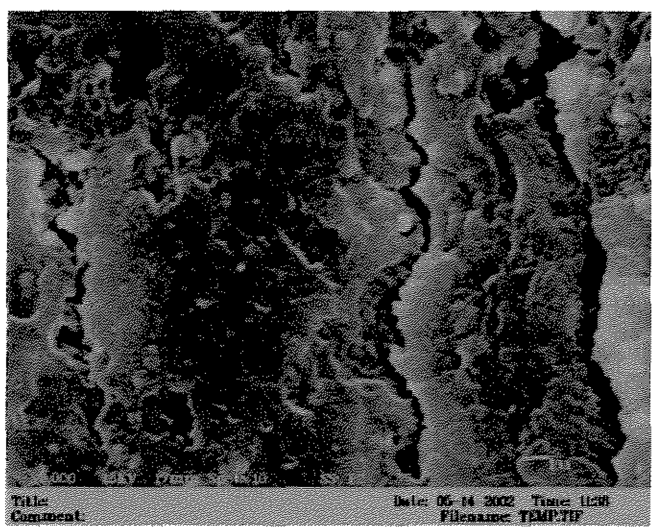

Figure 3. B- SEM micrograph of cohesive failure in adhesive present in control group (G1). C- SEM micrograph of cohesive failure in composite present in all groups.

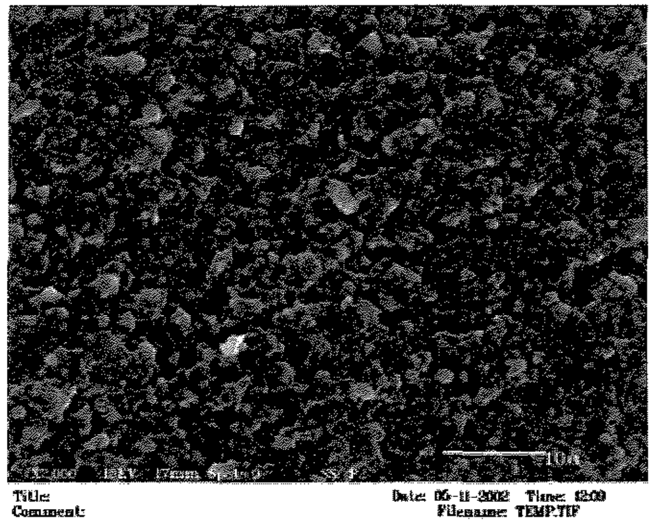

Figure 3. C- SEM micrograph of cohesive failure in composite present in all groups.

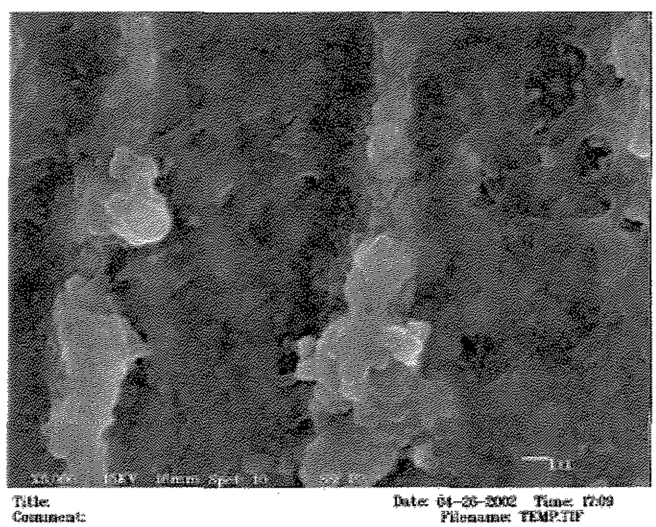

Figure 4. A- High magnification of the interphase failure mode at dentin side. The parallel direction of the tubule and tags can be observed.

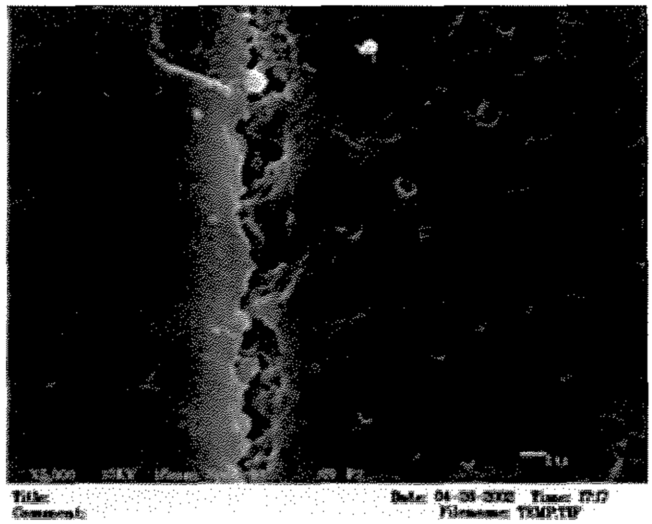

Figure 4. B- High magnification of the interphase failure mode at restoration side. The parallel direction of the dentin tubules can be observed through grooves at the interface of fracture.

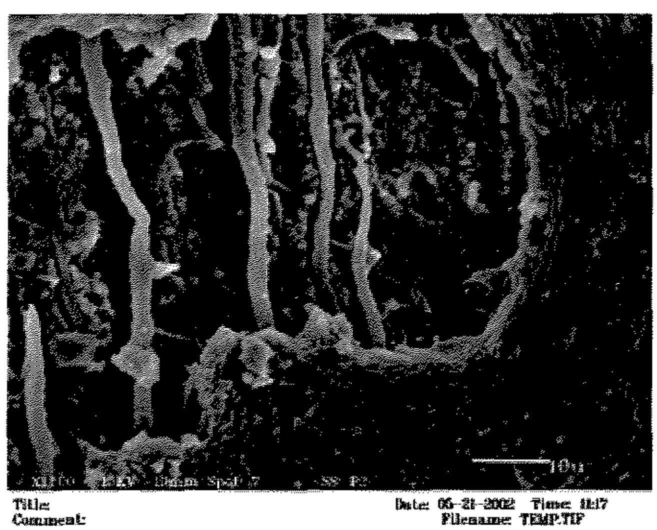

Figure 5. A- Fracture at the interphase shows tags in parallel directions.

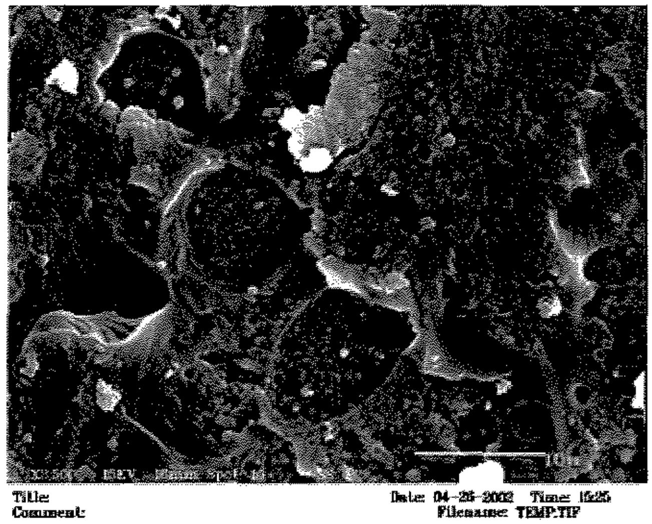

Figure 5. B- Fracture at the interphase shows tags in parallel directions. B- SEM micrograph of voids within the interphase observed in several specimens.

CAPÍTULO 3

Influence of thermal and mechanical load cycling on nanoleakage of class II restoration

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Short Title: Influence of thermal and mechanical cycling on nanoleakage

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Abstract

Purpose: To evaluate the effect of thermal and mechanical cycling on the degree and pattern of nanoleakage on cervical margins of class II restorations.

Material and Methods: Forty box-type class II cavities were prepared on bovine incisors. The cavities were restored with Single Bond and Z-250 composite resin (3M-ESPE) according to manufacturer's instructions. The teeth were randomly assigned to 4 groups: G1- Control, G2- Thermal cycling (2,000 cycles, 5-55°C), G3- Mechanical load cycling (100,000 cycles - 50 N), G4- Thermal and mechanical cycling group (2,000 cycles 5-55°C/ 100,000 cycles - 50 N). The specimens were then sealed leaving a 1mm window around the cervical margin interface. Samples were immersed in a 50% w/v ammoniacal silver nitrate for 24 hours, and exposed to a photodeveloper solution for 8 hours. Specimens were sectioned longitudinally, embedded in epoxy resin, polished and mounted on stubs, gold sputter coated and characterized in SEM using backscattered electron mode. Silver particle penetration length was measured directly on the SEM monitor and calculated as the percentage of the total length of cut dentin surface that was penetrated by silver nitrate. The data were analyzed by ANOVA and Fisher's PLSD test ($p < 0.05$).

Results: The degree of nanoleakage significantly increased when thermal and mechanical cycling was performed to the same specimens when compared to the other groups ($p < 0.05$). No differences were observed between the control,

thermal cycling, and mechanical cycling groups. No difference in nanoleakage pattern was observed between the groups.

Conclusion: Thermal and mechanical cycling combined, adversely affected nanoleakage values. Simulation of the oral condition might be crucial to better evaluate and understand the performance of adhesive materials.

Introduction

The major goal of successful restorative treatment is the effective replacement of tooth structure. In order to prevent deterioration of the sealing between material and tooth structure, the interface must resist dimensional changes (Mello *et al.*, 1997). Even after controlling the effects of polymerization shrinkage, deterioration of the restoration could occur subsequently, due to chemical, thermal, and mechanical load stresses (Jorgensen *et al.*, 1985; Abdalla & Davidson, 1993; Abdalla & Davidson, 1996; Mello *et al.*, 1997). It is important to establish a methodology using different types of stress, since the constant and rapid evolution of adhesive materials does not allow for long-term clinical trials. In this way, the use of mechanical and thermal cycling would allow for *in vitro* clinical simulation for evaluation of dental materials (Mello *et al.*, 1997; Adballa & Davidson, 1996).

The use of thermal cycling is frequently included in laboratory studies evaluating microleakage (Darbyshire *et al.*, 1988; Prati *et al.*, 1994; Alani & Toh, 1997; Hakimeh *et al.*, 2000) and more recently in nanoleakage evaluation (Li *et al.*, 2002a). The effectiveness of this method in altering the restoration interface has been questioned (Alani & Toh, 1997; Hakimeh *et al.*, 2000). Several studies suggest that occlusal mechanical cycling could accelerate the deterioration of the dentin/restoration interface (Darbyshire *et al.*, 1988; Abdalla & Davidson, 1993; Abdalla & Davidson, 1996; Mello *et al.*, 1997). Sealing ability evaluation (Abdalla & Davidson, 1993; Prati *et al.*, 1994; Abdalla & Davidson, 1996; Mello *et al.*, 1997; Hakimeh *et al.*, 2000; Li *et al.*, 2002b) has included mechanical load cycling in experimental protocols.

The evaluation of sealing ability of different materials can be made using microleakage tests and more recently nanoleakage tests. Nanoleakage has been used to describe microporous zones beneath or within hybrid layers that permitted tracer penetration to occur in the absence of interfacial gaps (Sano *et al.*, 1995). The advantage of the nanoleakage test as compared to the microleakage test is that detection of failure of an optimal seal can be made without the necessity of gap formation, while microleakage detects leakage in the presence of gaps. Nanoleakage, which is currently used to describe the pathway for degradation of a bonded interface, either in incompletely cured adhesive resin, within the hybrid layer, and/or demineralized dentin, can also be used to evaluate areas of a bonded interface that are not necessarily at a restoration margin (Sano *et al.*, 1995).

Since limited information exists on the effect of different oral stresses on nanoleakage of Class II cavity preparations, the aim of this study was to evaluate the influence of mechanical and thermal cycling on nanoleakage at the cervical margins of proximal slot restorations. The null hypothesis tested was that there was no influence of thermal and mechanical cycling on the degree and pattern of nanoleakage.

Materials and Methods

1. Specimen preparation

Forty bovine incisors were selected, cleaned of debris with curettes and pumice paste at slow-speed and stored in a 0.1% sodium azide saline solution. Occlusal surfaces were cut at the level of marginal ridges under water refrigeration, preparing a flat surface 4 mm above the cementum-enamel junction.

Class II slot preparations were prepared on the mesial surface following the dimensions: 3 mm of width, 5 mm of height (starting at the marginal ridge and with gingival margins in dentin) and 1.5 mm of depth towards the pulp chamber. All cavities were prepared using carbide burs (#245 KG Sorensen, Barueri, SP, Brazil) mounted in a high-speed handpiece under water refrigeration. The burs were replaced after every 5 preparations.

Cavities were restored with Single Bond adhesive system (3M-ESPE Dental Products, St. Paul, MN, USA) and composite resin Z-250 (3M-ESPE Dental Products, St. Paul, MN, USA). The adhesive system was applied according manufacturers' instructions: acid etch for 15 seconds, rinse for 15 seconds, blot dry, apply two consecutive coats of the adhesive, lightly air dry, and light-cure for 10 seconds. The preparation was filled with a micro-hybrid composite resin Z-250 in two horizontal increments and light-cured for 40 seconds each. A 1 mm overfill was left on the occlusal surface to enable mechanical load cycling on the restoration only. During all restorative procedures the light intensity (Optilux/Demetron/Kerr Corp., Orange, CA, USA) was measured periodically by a radiometer (Demetron/Kerr Corp., Orange, CA, USA) and ranged from 520 to 560 mW/cm^2. After the restorative procedure, the specimens were stored in distilled water at 37°C for 24 hours. Afterwards, they were finished and polished with Al_2O_3 abrasive discs (Sof-Lex Pop-on/3M Dental St. Paul, MN, USA). The teeth were then randomly divided into 4 groups:

G1= control group (no thermal and mechanical load cycling)

G2= thermal cycling only (2,000 cycles, 5-55 °C)

G3= mechanical load cycling only (100,000 cycles/ load = 50 N)

G4=thermal cycling (2,000cycles, 5-55 °C) and mechanical cycling (100,000 cycles/ load = 50 N)

The specimens subjected to mechanical load cycling had part of their roots embedded in epoxy resin (Buehler Ltd. Lake Bluff, IL, USA 60044) in order to obtain a flat occlusal surface perpendicular to the long axis of the tooth.

2. Thermal cycling and mechanical load cycling procedure

Specimens from groups G2 and G4 were subjected to 2,000 cycles in a thermocycling apparatus (MCT2 - AMM - 2 INSTRUMENTAL, Sao Paulo, SP, Brazil) with two baths at 5 ± 2°C and 55 ± 2°C respectively, with a dwell time of 60 seconds and a transfer time of 7 seconds between each bath.

Specimens from groups G3 and G4 were subjected to mechanical load cycling. The cyclic mechanical loading device used was a Leinfelder Wear Test Apparatus (custom by Dentsply/Caulk Technical Research, Milford, DE, USA), modified for loading test. The apparatus consisted of 4 stainless pistons in which a polyacetal cylinder tip was attached to the end. The polyacetal tips were placed in contact with the restoration. The loading device delivered an intermittent axial force of 50 N at 3 cycles/sec totaling 100,000 cycles (Figure 1). For group G4, thermal cycling was performed first and the mechanical cycling was performed after the thermal cycling was completed.

3. Immersion in dye and nanoleakage evaluation

After thermocycling and mechanical load cycling, the apices and the occlusal portion were filled with wax. The entire surface of each tooth was then coated with 2 layers of acid resistant varnish, except for a 1 mm width around the cervical margin. The teeth were immersed in a 50% ammoniacal silver nitrate solution for 24 hours (Tay *et al.*, 2002). The tracer solution was prepared by dissolving 25g of silver nitrate crystals (Sigma Chemical CO., St. Louis, MO, USA) in 25 ml of distilled water. Concentrated (28%) ammonium hydroxide (Sigma Chemical Co.) was used to titrate the black solution until it became clear. The solution was diluted to 50 ml with distilled water to achieve a 50% solution (pH=9.5). The teeth were then thoroughly rinsed in distilled water and immersed in a photo-developer solution for 8 hours under a fluorescent light.

After being thoroughly washed under tap water, the teeth were sectioned longitudinally, in a mesio-distal direction through the center of the restoration with a slow speed diamond wafering blade (Buehler-Series 15LC Diamond, Lake Bluff, IL, USA) and constant water coolant.

A total of 20 sections were obtained for each group. All the sections were embedded in epoxy resin (Buehler, Lake Bluff, IL, USA) and polished with Silicon carbide papers of ascending grits #600, 800, 1200 (Buehler Ltd.) and diamond pastes [6, 3, 1 μm (BuehlerLtd.)]. Specimens were then ultrasonically cleaned, air dried, mounted in stubs, left to rest for 24 hours, gold sputter coated (Polaron E-5200 Energy Beam Sciences, Agawan, MA, USA), and examined in a scanning electron microscope (model 6500, JEOL Corp., Peabody, MA, USA) using backscattered electron mode and second beam mode.

The length of silver nitrate penetration along the cervical wall was measured using different magnifications, permitting localization of the end of the silver nitrate trace. The leakage from each restoration was calculated as the percentage of the total length of cut dentin surface that was penetrated by silver nitrate, i.e. the ratio of the length of silver nitrate penetration along the cervical dentin / resin interface and the total length of the cervical restoration wall. Statistical analysis was performed using ANOVA and Fisher's PLSD test ($p < 0.05$).

Results

The mean nanoleakage length and standard deviations for each group are described in Table 1. One-way ANOVA revealed statistically significant difference between Group 4 (thermal and mechanical cycling) and Groups 1 (control), 2 (thermal cycling), and 3 (mechanical cycling) ($p < 0.05$). Nanoleakage significantly increased when thermal and mechanical cyclings (G4) were performed on the same specimens when compared to the other groups (G1, G2, and G3). No differences were observed between the control, thermal cycling, and mechanical cycling groups.

The SEM evaluations using backscattered mode offered more accurate visualization of the silver particles (Figure 2 A and B) when compared to the secondary electron beam mode. Therefore, all the nanoleakage analysis were done with the backscattered mode. Two nanoleakage patterns were observed: silver was either present at the bottom of the hybrid layer (Figure 3), or between the bottom of the adhesive or top of the hybrid layers (Figure 4). No difference in nanoleakage pattern was observed between the groups, except that mechanical cycling groups (G3 and G4) presented a higher deposition of silver particles at the bottom and top of the hybrid layer (Figure 5).

Discussion

The durability of bond between adhesive resins and dentin is of critical importance for the longevity of bonded restorations (Okuda *et al.*, 2001). It is common to have the margins of cavities on dentin due to the extensive loss of tooth structure by decay. The dentin substrate remains a challenge when trying to achieve a reliable bonding and sealing ability, due to the complex characteristics of the substrate. In this study, bovine teeth were used in place of human teeth (Nakamichi *et al.*, 1983; Reeves *et al.*, 1995), which presents major advantages with the possibility of standardizing age, sclerosis, and amount of wear.

The use of thermal and mechanical load cycling has been recommended for aging of restorations (Abdalla & Davidson, 1993; Abdalla & Davidson, 1996; Mello *et al.*, 1997; Alani & Toh, 1997; Hakimeh *et al.*, 2000). The *in vitro* effect of thermal cycling in microleakage has been thoroughly investigated (Darbyshire *et al.*, 1988; Prati *et al.*, 1994; Alani & Toh, 1997; Hakimeh *et al.*, 2000), however few studies have reported its effect on nanoleakage (Dorfer *et al.*, 2000; Li *et al.*, 2002a). The quantity of cycles and the temperatures used seem to be the major difference among different studies (Prati *et al.*, 1994; Alani & Toh, 1997; Hakimeh *et al.*, 2000). Controversial results have been reported regarding the influence of thermocycling on microleakage (Darbyshire *et al.*, 1988; Prati *et al.*, 1994; Chan & Glyn-Jones, 1994; Hakimeh *et al.*, 2000) and non-influence for nanoleakage measurements (Dorfer *et al.*, 2000; Li *et al.*, 2002a).

The use of 2000 cycles and temperatures of 5-55^{o}C were chosen as an average of the number of cycles employed in different studies, and the use of

ISO standardization bath temperatures allows for comparison among studies. In the present study, thermal cycling performed alone did not have any effect on nanoleakage values. Although this data is in accordance with other studies, these studies used different numbers of cycles and bath temperatures (Dorfer *et al.*, 2000; Li *et al.*, 2002a;). In addition, thermal cycling was performed on flat surfaces and in class V restorations, which differed from the present study. The use of different cavity configurations and flat surfaces has implications in regard to polymerization shrinkage, and bond strength studies have shown differences in values according to the C-factor (Feilzer *et al.*, 1987; Bouillaguet *et al.*, 2001).

The use of mechanical load cycling has been studied due to the potential capability of simulating mastication. In the present study the use of a cylindrical polyacetal tip touching only the material aimed to fatigue the restoration. It is difficult, if not impossible, to simulate the occlusal forces, due to the variation of age, sex, and type of tooth. These factors interfere with the force employed. However, the simulation of a mean force is necessary for further comparison between studies. The force of 50 N was chosen as a medium low force present during mastication (Anderson, 1956) which when employed with high number of cycles, 100,000 used in this study, can promote a continuous stroke aimed to fatigue primarily the restoration interface.

For microleakage evaluation, controversial results related to the effect of load cycling have been reported (Abdalla & Davidson, 1993; Prati *et al.*, 1994; Mello *et al.*, 1997; Ausiello *et al.*, 1999; Hakimeh *et al.*, 2000). In the present study no statistically significant influence was observed when only mechanical load cycling was performed. Li *et al.* (2002b) did not find any effect of load cycling on nanoleakage on both flat surface and class V Cavities restorations. Although their results are in accordance those of the present study, class II restorations were used, so the concepts of C factor (Feilzer *et al.*, 1987) due to different cavity configuration and consequently polymerization shrinkage are different, and could have some implication on the results.

In the present study, mechanical cycling and thermal cycling did not have any effect on nanoleakage values when applied alone; however, the association of both significantly increased the length of silver nitrate penetration. Nara *et al.* (2002) observed that the association of thermal and load cycling was effective to examine microleakage of cervical composite restorations. Bedran de Castro *et al.* (unpublished data) evaluating microtensile bond strength on cervical walls of class II cavities preparations, performed in the same condition and distributed in the same groups of the present study, found a decrease on bond strength values when thermal and mechanical load cycling were performed in the same specimens. According to their study, the use of mechanical load cycling resulted in a change of fracture mode pattern with an increase in mixed failures.

In the present study, two types of nanoleakage patterns were observed, tracer leakage at the bottom of the hybrid layer (Sano *et al.*, 1995), and between the bottom of the adhesive and top of the hybrid layers (Li *et al.*, 2000). In our study, an ammoniacal silver nitrate solution was used (Tay *et al.*, 2002). Tay *et* *al.* (2002) using this silver nitrate modified solution observed two types of nanoleakage patterns with TEM, the reticular pattern present in the hybrid layer which is commonly found, and also a spotted pattern that probably represents regional hydrophilic phases within the adhesive systems' matrices that are more prone to water absorption. The reticular mode of the nanoleakage pattern, in particular the silver deposits that were oriented perpendicular to the surface of the hybrid layer, is the morphological manifestation of water treeing (Tay *et al.*, 2002). These phenomena were observed for self-etching (Tay *et al.*, 2002) and total etching adhesive systems (Tay & Pashley, unpublished data). Tay *et al.* (2002) speculated that it represents a region in which bulk water is retained within the adhesive dentin interface.

Thicker layers of Single Bond prevent proper evaporation of the solvent, leading to poor polymerization and a decrease in bond strength (Zheng *et al.*, 2001). In addition, the use of a moist bonding technique in cavity preparations makes blot drying a critical step, and the presence of excess water along line angles may compromise full evaporation of the solvent. It is difficult to remove the last traces of water from ethanol based adhesive systems, due to the increased capacity of ethanol to form hydrogen bonds with water (Tay *et al.*, 2002). We suggest that the presence of water voids at the adhesive layer and/or interfase layer (Nakabayashi & Pashley, 1998) could also contribute to the formation of the treeing pattern present at the interface-adhesive layer.

In the present study, an increase in nanoleakage length was observed only for the thermal and mechanical load cycled group (G4). No differences in the pattern of nanoleakage was observed among the groups (G1, G2, G3, and G4), except that for mechanically cycled (G3) and thermally/mechanically cycled (G4), the deposits of silver nitrate crystal were larger. According to our data we can not affirm what took place at the bonding site, but we can suggest that thermal cycling may induce some changes at the resin-adhesive-dentin interface, based on differences in the different coefficients of thermal expansion between the adhesive materials and the tooth structure, since it is a small restoration. This effect on the bonding may contribute to the effectiveness of mechanical load cycling in fatiguing the restoration through the weakest points present (i.e. water and adhesive voids, unprotected collagen present in the demineralized dentin zone, and water treeing).

Weakening of the adhesive resin due to mechanical loading is a very important issue in restorative dentistry, since the demand of posterior composites by patients has significantly increased. Further studies should be done evaluating the effect of mechanical and thermal cycling on different adhesive systems and also different dentin substrates (caries affected vs. sound dentin, deep vs. superficial dentin, parallel and perpendicular directions of dentin tubules) using nanoleakage evaluations.

Conclusion

The null hypothesis that thermal cycling and mechanical load cycling would not influence bond strength was rejected when both stresses were used on the same specimens, increasing the length of nanoleakage penetration.

Thermal and mechanical cycling combined, adversely affected nanoleakage values. Appropriate simulation of the oral condition might be crucial to better evaluate and understand the performance of adhesive material using laboratory tests.

Acknowledgment

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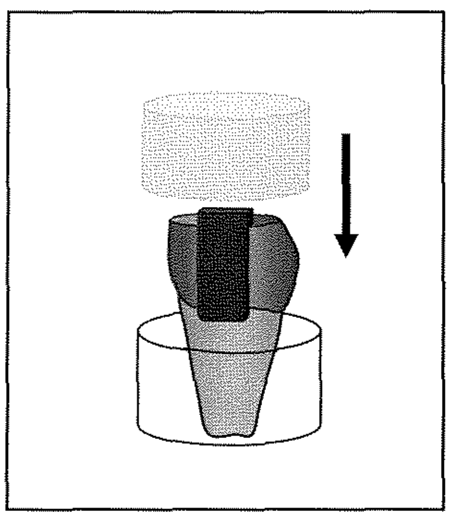

Figure 1. Illustration of the load application on the occlusal surface. Black arrow indicates direction of loading force.

Table 1. Means and standard deviation for nanoleakage values.

Groups	*Thermal cycling (2,000 cycles/5-55°C)*	*Mechanical cycling (100,000 cycles/50 N)*	*Mean (SD) (%)*	*$p<0.05$*
G1	-	-	22.26 (15.89)	b
G2	+	-	22.91 (24.55)	b
G3	-	+	28.82 (22.63)	b
G4	+	+	50.22 (25.56)	a

(+ = performed; - = not performed)

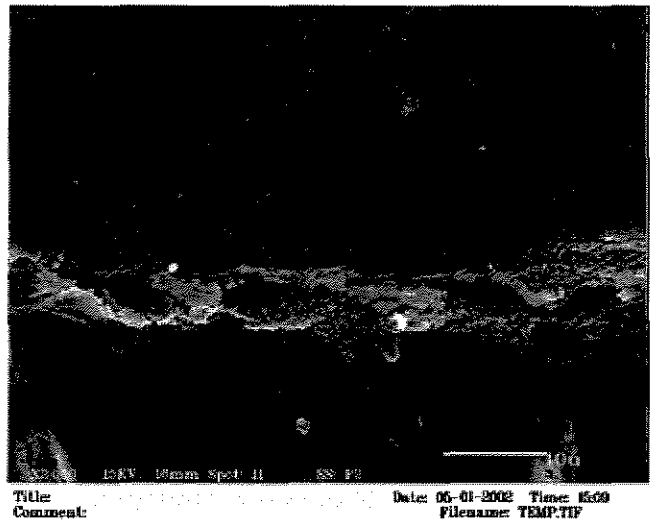

Figure 2. A- SEM Secondary beam micrograph of the dentin/restoration interface.

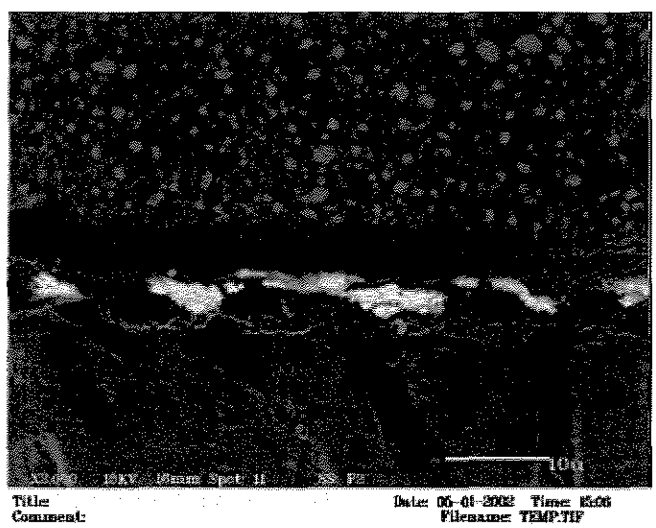

Figure 2. B- SEM backscattered micrograph of the dentin/restoration interface. The backscattered mode offers more accurate visualization and analysis of the silver nitrate deposition.

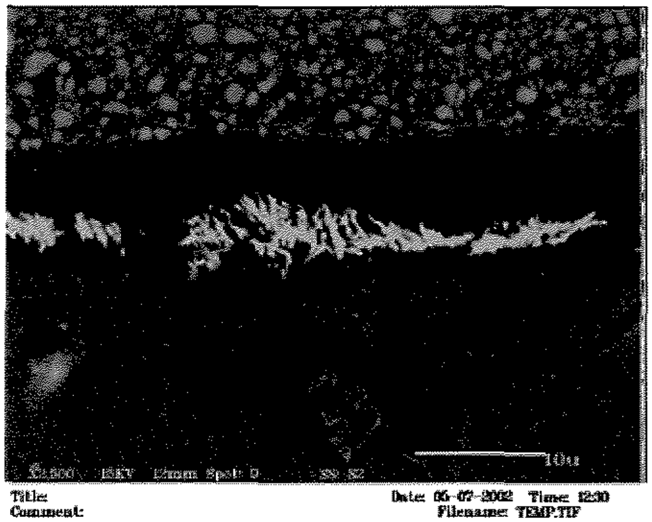

Figure 3. SEM micrograph of the interface shows silver nitrate particle deposition at the bottom of the hybrid layer.

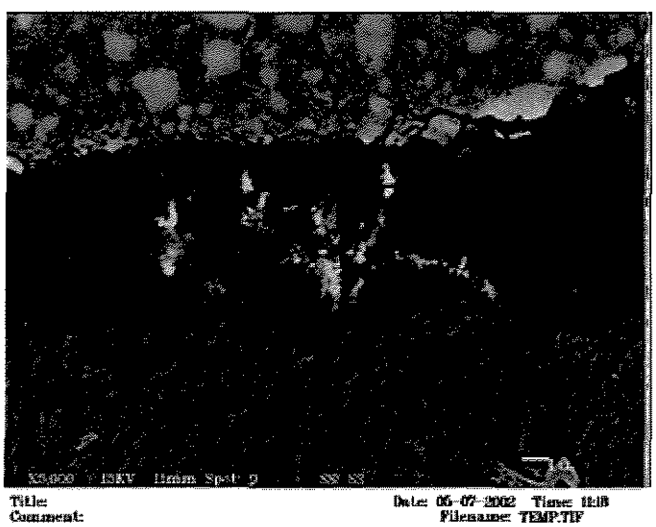

Figure 4. A- SEM micrograph of the interface shows silver nitrate particle deposition at the adhesive layer and hybrid layer.

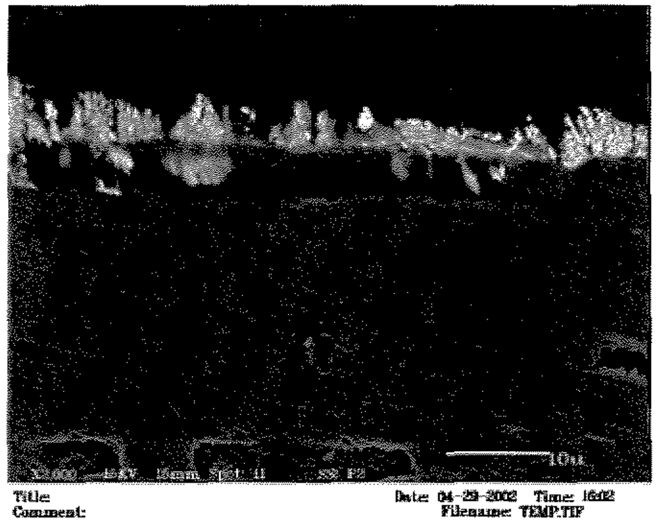

Figure 4. B- SEM micrograph of the interface showing silver nitrate particle deposition at the body and top of the hybrid layer.

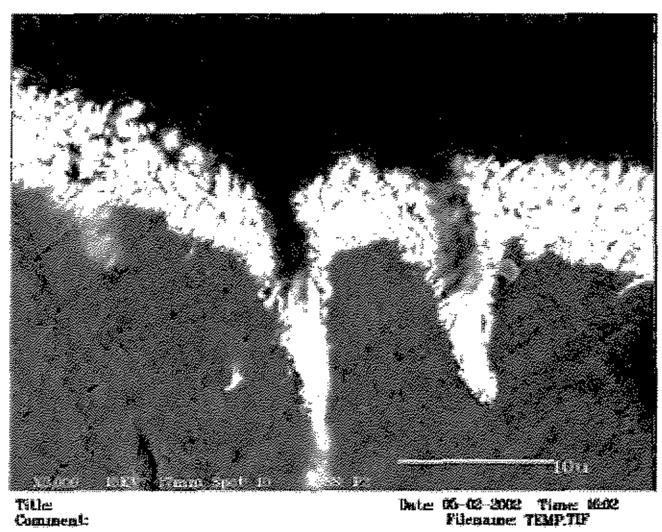

Figure 5. SEM micrograph shows high deposition of silver particles at the bottom of the hybrid layer, observed for mechanical load cycling specimens.

CAPÍTULO 4

Long-Term Microtensile Bond Strength of Restorations Subjected to Thermo-Mechanical Load Cycling

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Long Term Microtensile Bond Strength of Restorations Subjected to Thermo-Mechanical Load Cycling

Abstract

Objective: To evaluate the long term effect of thermal and mechanical cycling on dentin bond strength to cervical margins of class II restorations. ***Material and Methods***: Sixty class II slot cavities were prepared in bovine incisors. The cavities were restored with Single Bond and Z-250 composite according to manufacturer's instructions. The teeth were then divided into 2 groups (n=30): specimens that would receive thermo-mechanical load cycling (2,000 cycles 5-55°C/ 100,000 cycles; 50N) (TM) and the control (C). Fifteen specimens from each group were tested at baseline and the remaining 30 specimens were stored in distilled and deionized water at 37 °C and tested after one year. For microtensile evaluation, the restorations were sectioned perpendicular to the cervical bonded interface into 0.7 ± 0.2 mm thick slabs. The slabs were further trimmed at the interface to 1.4 ± 0.2 mm with a fine diamond bur to produce a cross-sectional surface area of ca.1mm^2. Specimens were then subjected to microtensile bond testing. The bond strength data were analyzed using two-way ANOVA and Fisher's PLSD test ($p<0.05$). Fracture mode analysis was performed using SEM. ***Results***: At baseline, bond strength of the TM group was significantly lower when compared to the C group (p= 0.012). However, after one year storage, a significant decrease in bond strength was observed for the C group compared to baseline. No significant differences were noted

between the C and TM groups at one year. No interaction was observed between groups (C and TM) and storage time ($p=0.098$). For the fracture mode evaluation, at baseline, mixed failure was predominant for the C group, and increased after TM. Decrease in mixed failure was observed after one year storage with concomitant increase of interphase failure.

Clinical Significance: Dentin bond strength decreased after one year storage in water at 37°C. The combination of thermal and mechanical cycling adversely affected bond strength at baseline similar to the control at one year, and could thus be used as an accelerating aging method.

Introduction

Most of the currently marketed adhesives achieve high bond strength after polymerization of the resin (1). However, the longevity of the adhesive bond is still an area of current interest in adhesive dentistry. Long-term studies have been conducted both *in vivo* and *in vitro* in order to better understand the performance of adhesive systems. Several *in vitro* studies report a decrease in bond strength over time (2, 3, 4, 5) as well as an increase in nanoleakage (4). *In vivo* studies conducted in monkeys have reported no effect of time on bond strength after one year evaluation (6, 7), however, an increase in hybrid layer and adhesive resin porosity has been detected (6).

The use of thermal and mechanical stresses has also been used in *in vitro* studies in order to mimic the natural aging process of the restoration. Studies evaluating microleakage have shown conflicting results regarding the effectiveness of both mechanical (8, 9, 10, 11) and thermal stresses (11, 12). Very limited bond strength studies have been conducted associating these stresses (13, 14, 15, 16). The establishment of *in vitro* methodologies using different types of stresses present in the oral environment is crucial, since the constant and rapid evolution of adhesive materials does not easily allow for long-term clinical trials.

With the recently developed microtensile bonding test (17), measurement of bond strength to small surface areas (0.5-1.0mm^2) have become possible. It permits the testing of irregular surfaces and cavities, which are more clinically relevant than flat surfaces, but were not testable with conventional shear and tensile bond tests. The benefits of evaluating small areas such as cervical, gingival, and axial walls as well as the pulpal floor of Class I, II, V preparations are substantial and closer to the clinical situation, since clinicians usually do not bond to flat surfaces with a very small C-factor.

Our previous study (16) has shown that the association of thermo-mechanical load cycling resulted in decrease of bond strength values; however, no long term effect of mechanical and thermal load cycling on bond strength was reported. Therefore, the aim of this study was to evaluate the long term effect of mechanical and thermal cycling on microtensile bond strength (μTBS) and failure mode pattern at the cervical margins of class II slot restorations. The null hypothesis tested was that long term storage would not affect bond strength and fracture modes.

Materials and Methods

Sixty bovine incisors were selected, cleaned of debris with curettes and pumice paste in a slow-speed handpiece, and subsequently stored in a 0.1% sodium azide saline solution. Occlusal surfaces were cut at the level of marginal ridges under water irrigation, preparing a flat surface 4 mm above the cementum-enamel junction.

Class II slot preparations were prepared on the mesial surface with the following dimensions: 3 mm wide, 5 mm high (starting at the marginal ridge and with gingival margins in dentin) and 1.5 mm deep towards the pulp chamber. All cavities were prepared using carbide burs #245[a] mounted in a high-speed handpiece under water irrigation. The burs were replaced after every 5 preparations.

Cavities were restored with Single Bond adhesive system [b] and composite resin Z-250 [b]. The adhesive system was applied according manufacturer's instruction: acid etch for 15 seconds, rinse for 15 seconds, blot dry, apply two consecutive coats of the adhesive, lightly air dry, and light-cure [c] for 10 seconds. The preparation was filled with a micro-hybrid composite resin Z-250 [b] in two horizontal increments and light-cured for 40 seconds each. A 1 mm overfill was left on the occlusal surface to enable mechanical load cycling on the restoration only. During all restorative procedures the light intensity [c] was measured periodically by a radiometer [c] and ranged from 520 to 560 mW/cm^2. After the restorative procedure, the specimens were stored in distilled and deionized water at 37°C for 24 hours. Afterwards, they were finished and polished with Al_2O_3 abrasive discs Sof-Lex Pop-on [b]. The teeth were then randomly divided into 2 groups (n=30):

C -Control group (no thermal and mechanical load cycling)

TM -Thermal cycling (2,000 cycles, 5-55 °C) and mechanical cycling (100,000 cycles / load = 50N)

The specimens subjected to mechanical load cycling had part of their roots embedded in epoxy resin [d] in order to obtain a flat occlusal surface perpendicular to the long axis of the tooth.

Thermal and mechanical load cycling procedure

Specimens from the TM group were subjected to 2,000 cycles in a thermocycling apparatus [e] with two baths at 5 ± 2 °C and 55 ± 2 °C each with a dwell time of 60 seconds and a transfer time of 7 seconds between each bath. Then they were subjected to mechanical load cycling in a Leinfelder Wear Test Apparatus [f], modified for loading test. The apparatus consisted of 4 stainless pistons in which a polyacetal cylindrical tip was attached to the end. The polyacetal tips were placed in contact with the restoration. The loading device delivered an intermittent axial force of 50 N at 3 cycles/sec totaling 100,000 cycles (Figure 1-A).

Microtensile Bonding Test

Half of the number of specimens from each group was tested after thermo-mechanical load cycling. The other half was kept immersed in distilled and deionized water at 37°C for one year and then tested for microtensile strength. The distilled and deionized water was replaced weekly.

Before testing, a 3 mm-thick resin block was built on the outermost surface of the restoration in order to produce grips for the microtensile bond test. The restorations were sectioned perpendicular to the cervical bonded interface of each tooth into 0.7 ± 0.2 mm thick slabs (n = 2 per restoration) with a slow speed diamond wafering blade (Series 15LC Diamond [d]) and constant water irrigation (Figure 1-B). The composite/dentin interface was further trimmed at the interface to 1.4 ± 0.2 mm with a fine diamond bur to produce a cross-sectional surface area of ca.1mm^2 (Figure 1-B). All specimens were then glued with Zapit [g] to a Ciucchi's jig which was mounted on an universal testing machine [h] and subjected to microtensile bond testing at a cross-head speed of 1 mm/min. Means and standard deviations were calculated and expressed in MPa. Statistical analysis was performed using ANOVA and Fisher's PLSD test ($p < 0.05$).

Fracture mode analysis

For fracture mode analysis, specimens were stored in 10% neutral buffered formalin solution after debonding for at least 8 h. All specimens were then mounted on stubs, gold sputter coated [i], and examined in a scanning electron microscope [j]. Fracture modes were classified as: failure between the deepest portion of the adhesive resin and the top layer of the demineralized dentin (Interphase) (18), cohesive failure in composite, cohesive failure in adhesive and mixed failure (association of two or more failures). Fractures were calculated according to the percentage present in each specimen.

Results

1. Microtensile bond strengths

Microtensile bond strength values for the groups are described in Table 1. Two- way ANOVA showed no interaction between groups (C and TM) vs. storage time ($p = 0.098$). At baseline, a statistically significant decrease ($p = 0.012$) in μTBS was observed for the thermo-mechanical load cycling group (TM) as compared to the control group (C). No statistically significant differences were observed between control group and thermo-mechanical load cycling group after one year storage ($p = 0.76$). A comparison between baseline and one year storage pointed to a significant decrease in μTBS values for the control group after one year ($p = 0.005$), however the storage time did not influence the results of the thermo-mechanical load cycling group.

2. Fracture mode analysis

The fracture mode analysis results are shown in Figure 2. Failure at the interphase was observed mostly for the control and thermo-mechanically challenged groups after 1 year storage (Figure 3 A). Interphase failure after 1-year storage presented very porous surface for either C and TM as compared to the baseline C and TM groups (Figure 3 B). Adhesive and cohesive failures appeared in a very low percentage and were only observed for groups C and TM at baseline. Mixed failure was present in all groups but in different percentages (Figure 2). Evaluation of baseline specimens disclosed predominantly mixed failure (interphase and cohesive composite/dentin), which increased after thermo-mechanical load cycling. A decrease in mixed failures was observed after one year storage, with a concomitant increase in interphase failures. Fracture at the interphase, showing the tags running parallel to the bonded interface (Figure 4), was present in all groups tested, but at a higher incidence and extent for the specimens stored for one year, indicating an increase in hybrid layer failure.

Discussion

The durability of bonds between adhesive resins and dentin is of crucial importance for the longevity of bonded restorations. The dentin substrate remains a challenge when trying to achieve reliable bonding and sealing, due to the complex characteristics of the substrate. Sealing ability (nanoleakage and microleakage) and bond strength (tensile and shear bond strength) are the two main types of laboratory tests used to evaluate the performance of restorative systems. The feasibility of using these tests to evaluate restorations placed in cavity preparations permits the evaluation of a specific material in a condition that is clinically relevant. In the present study, bovine teeth were used in place of human teeth (19, 20), which has the major advantages of possibly standardizing age, sclerosis, and amount of wear.

Under the conditions of this study, the long term storage of class II composite restorations resulted in a decrease of bond strength of the control group after one year storage. Thermo-mechanically challenging specimens significantly decreased bond strengths at baseline as compared to the control group. However, the one-year results of the TM group remained statistically similar to its respective baseline group. This may indicate that thermo-mechanically challenging adhesive restorations could result in simulating long-term storage conditions.

The time dependent degradation of bond strength has been studied and according to different studies it may result from the plasticizing effects of water on resin and collagen (21); from water sorption and/or hydrolysis of adhesive resin (3, 5, 6); and/or from hydrolysis of collagen fibrils at the base of the hybrid layer (3).

The use of thermo-mechanical load cycling has been described as a method of aging restorations through the simulation of oral conditions. Thermal cycling simulation is the most widely used method in the evaluation of adhesive systems (10, 11, 22). The quantity of cycles and the temperatures used seems to be the major difference among different studies (10, 11, 22). Controversial results have been reported for the influence of thermocycling on microleakage (8-11), and little is known about the influence of thermal cycling on microtensile bond strength. Nikaido *et al.* (15) and Bedran de Castro *et al.*, 2002 (16) observed no influence of 2000 thermal cycles (5-55 ^{o}C) on μTBS of flat dentin surfaces and class II restorations, respectively.

The use of mechanical load cycling has been studied due to the potential capability of simulating mastication. As for thermal cycling studies, differences in the methodologies (load force, number of cycles, frequency of cycles, restorative material, factor C) can be responsible for the different results presented among studies using mechanical load cycling. Nikaido *et al.* (14) evaluated the influence of 50,000 cycles on class I preparations restored with self etching or total etch adhesive systems. However, they did not report the influence of mechanical load cycling alone on μTBS. Bedran-de-Castro *et al*, 2002 (16) reported no statistically significant difference on micro-tensile bond strength when mechanical cycling was applied. On the other hand the authors observed an adverse effect on μTBS when thermal cycling and load cycling were performed in the same class II restorations (16).

Nikaido *et al.*, 2002 (15) observed a decrease in microtensile bond strength of a self etching adhesive system when thermal and mechanical load cycling was performed concomitantly in class I restorations, using the same load force (50N) and number of cycles (100,000) employed in the present study.

In the present study, a force of 50 N (23) and 100,000 cycles was chosen in order to simulate a low and constant load found during mastication. This load cycling of 2000 thermal cycles at 5-55 °C has been shown to produce a decrease in μTBS (16). The present study confirmed these results observed for μTBS. The use of thermo-mechanical load cycling promoted a decrease in bond strength values that were statistically comparable with the results obtained for specimens stored one year. Even though the fracture mode analysis was different when comparing baseline thermo-mechanical cycling to one year storage, the association of thermal and mechanical load cycling has been shown as a potential method to accelerate the aging of restorations.

Several reports have evaluated the durability of dentin bonding *in vitro* and *in vivo*. In an *in vivo* study, Sano *et al*, 1999 (6) demonstrated that fractured interfaces of resin-dentin bonds obtained using a self-etching primer after one year of function exhibited increased porosity at the bonded interface and adhesive resin, whereas bond strength remained unaltered. They speculated that the increased porosity at the bonded interface over time might have occurred via nanoleakage pathways that developed within the hybrid layer. Takahashi *et al.* 2002 (7), observed in an *in vivo* study a tendency for decreased bond strength after one year of function while the analysis of the fracture interface showed a high porosity at the bottom of the hybrid layer over time. An *in vivo* study conducted by Hashimoto *et al.*, 2000 (24) revealed a decrease in bond strength over time (one-to-three years evaluation). Their SEM observations of the resin-dentin interface revealed the complete loss of resinous material between the collagen fibrils or depletion of collagen fibrils within the degraded hybridized dentin. The authors speculated the\at nanoleakage in the oral cavity was caused by deterioration of the hybrid layer creating nanometer-sized diffusion channels. A possible correlation between bond strength and nanoleakage was not observed for self-etching systems (5) and total-etching systems (4) after long term evaluation. Okuda *et al.*, 2002 (5) speculated that the decrease in bonding was due to hydrolysis of ester bonds of the polymerized resin within the hybrid layer that gradually increased over time as water diffused through nanoleakage channels, resulting in low bond strength and interfacial failure after nine months

In the current study, the fracture mode analysis clearly shows a difference between the fracture mode from baseline groups and one year storage groups. The increase in the incidence of interphase failure indicates a possible degradation of the adhesive layer and/or hybrid layer. The increase in the exposure of dentin tags (Figure 4) in parallel directions, of the specimens stored for 1 year, indicates a failure at the hybrid layer. Beside the differences in the incidence of the different types of fracture, a difference in the morphology of the fracture was evident. After one year storage, fracture at the interphase showed a very porous surface with no apparent adhesive as compared to the observed "glazed" surface at the baseline (Figure 3 A-B). Tay *et al.*, 2002 (25) using an ammoniacal silver nitrate solution observed two types of nanoleakage patterns with TEM; the reticular pattern often present in the hybrid layer and also a spotted pattern that probably represents regional hydrophilic phases within the adhesive systems' matrices that are more prone to water absorption. The reticular mode of the nanoleakage pattern, in particular the silver deposits that were oriented perpendicular to the surface of the hybrid layer, is the morphological manifestation of water treeing (25). These phenomena were observed for self-etching (25) and total etching adhesive systems (Tay and Pashley, unpublished data). The authors speculated that it represents a region in which bulk water is retained within the adhesive dentin interface.

Thicker layers of the adhesive system used in this study, Single bond, has been previously shown to prevent proper evaporation of the solvent, leading to poor polymerization and a decrease in bond strength (26). In addition, the use of a moist bonding technique in cavity preparations makes blot drying a critical step, and the presence of excess water along line angles may compromise full evaporation of the solvent. It is difficult to remove the last traces of water from ethanol based adhesive systems, due to the increased capacity of ethanol to form hydrogen bonds with water (25). In presence of water and/or solvent, polymerization of the monomer is compromised and the poor cured of the monomer can lead to rapid degradation of resinous matrix. We speculate that after long time storage, the existence of water treeing and/or water voids (16) at the hybrid layer and/or adhesive turn that interface more permeable to outside fluid accelerating the degradation through hydrolysis of the ester bonds of the polymerized resin (5), increasing the porosity and reducing the bond strength.

It is important to note that the thermo-mechanical load cycling group was not affected by long term storage. Although there was a trend for decreased bond strength after one year, the bond strength was not statistically different from the control group at the same time point. At baseline, the fracture mode between the control group and thermo-mechanical cycling group was different. An increase in mixed failures as compared to the control group at baseline may indicate a different pattern of stress distribution which could decrease bond strength. After one year storage, the fracture mode present from the two groups was very similar, presenting high percentage porosity of interphase failure. According to this data, we speculate that although the thermo-mechanical loading promoted a decrease in bond strength and fracture mode, the degradation observed after one year was not related to the thermo-mechanical load cycling. Future studies should focus on using thermo-mechanical load cycling after different storage times, to better determine a potential deterioration of the bonding after water degradation.

The null hypothesis that long term storage would not influence bond strength was rejected when comparing control group at baseline and after one-year storage. The ability of the adhesive resin resist to long term storage and simulation of oral conditions is a very important issue in restorative dentistry, since the demand for composite resins of both anterior and posterior teeth has significantly increased. Future studies should also focus on determining the durability of self-etching adhesive systems under mechanical loading.

a - KG Sorensen, Barueri, SP, Brazil

b - 3M-ESPE Dental Products, St. Paul, MN, USA

c - Optilux 501/Demetron/Kerr Corp. - Orange, CA, USA

d - Buehler Ltd. Lake Bluff, IL, USA

e - MCT2 - AMM - 2 INSTRUMENTAL, Sao Paulo, SP, Brazil

f - Custom by Dentsply/Caulk Technical Research, Milford, DE, USA

g - DVA, Corona, California USA

h - EZ Test, Shimazu Co., Kyoto, Japan

i - Polaron E-5200 energy Beam Sciences, Agawan, MA, USA

j - Model 6500, JEOL Corp., Peabody, MA, USA

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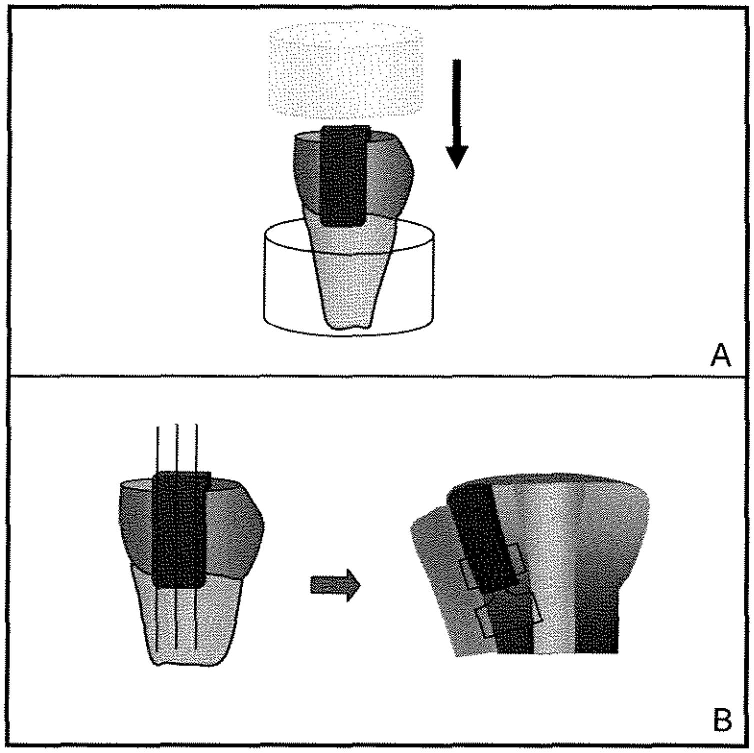


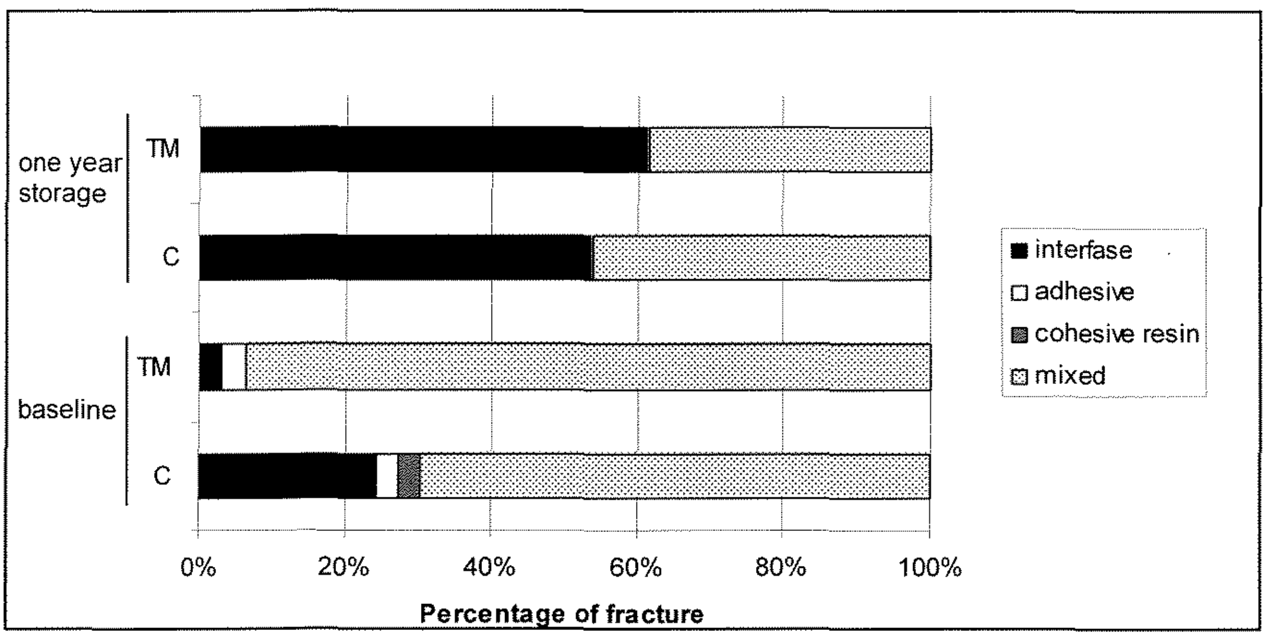
Figure 1. A- Illustration of the load application on the occlusal surface. B- Illustration of the restoration section and slab configuration for microtensile bond strength test.

Table 1. Mean and standard deviations for microtensile bond strength values.

Groups	*Storage time*	*Mean*	*SD*	*p<0.05**
Control (C)	***Baseline***	29.92	13.94	a
	One year	18.96	8.84	b
Thermo- mechanical Cycling (TM)	***baseline***	21.65	7.95	b
	One year	18.68	7.88	b

*Different letters indicate statistical differences between groups.

Figure 2. Incidence of fracture mode for the groups evaluated.



C= control group/ TM= Thermo-mechanical load cycling group.

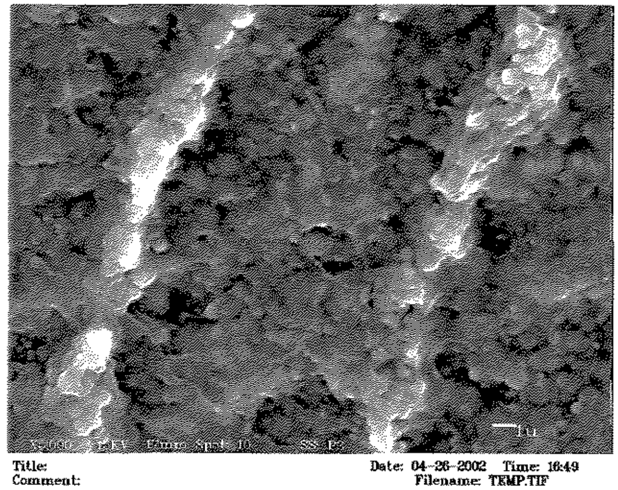


Figure 3. A- Representative high magnification micrograph (5000x) of interphase failure observed for baseline groups.

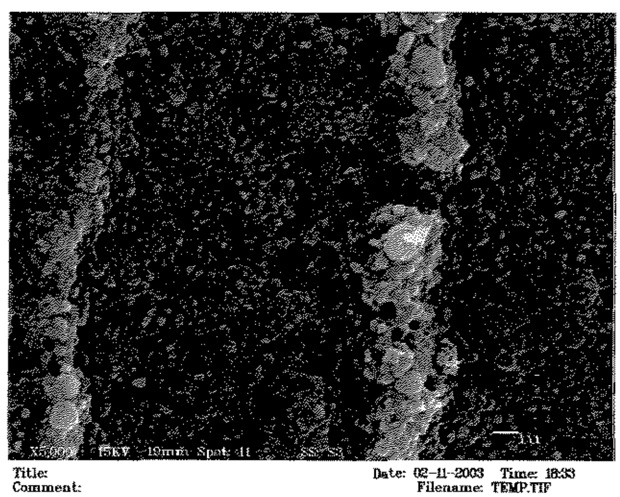


Figure 3. B- Representative high magnification micrograph (5000x) of interphase failure observed for one year storage groups. Increased porosity is observed after one year of storage.

Figure 4. Representative micrograph (1000x) of interphase fracture from the one year storage groups. Tags in parallel directions are exposed, demonstrating a potential weakness of the hybrid layer after one year of storage.

Considerações Gerais

Para a avaliação de materiais restauradores, o desenvolvimento de estudos clínicos pode proporcionar resultados definitivos e reais (Swift, Heymann, Perdigao, 1995). No entanto, devido ao constante desenvolvimento destes materiais, especialmente materiais que promovem adesão, a avaliação clínica se torna praticamente inviável uma vez que as chances são grandes destes materiais não mais estarem sendo comercializados. Desta maneira, estudos laboratoriais são de grande importância para a avaliação do comportamento destes diferentes materiais.

Atualmente, existe grande necessidade de que estudos "in vitro" sejam mais acurados possibilitando a simulação de condições encontradas clinicamente (Mello *et al.*, 1997). Assim, simulações de desafios químicos, mecânicos e térmicos têm sido estudados para inclusão durante a avaliação de materiais restauradores. O uso de ciclagem térmica foi preconizado há décadas atrás e sua indicação tem sido questionada (Alani & Toh, 1997; Hakimeh *et al.*, 2000; Kidd, 1976; MIYAZAKI *et al.*, 1998). Estudos têm mostrado sua efetividade em promover uma tensão na interface de adesão e conseqüentemente promover o aumento da microinfiltração marginal bem como diminuição da resistência da união. No entanto, é difícil comparar diretamente os resultados pois existe variação do material restaurador utilizado, principalmente quando se comparam estudos atuais com estudos desenvolvidos há 20 anos. Além disto, variações na metodologia para identificação (corante e/ou traçador químico), bem como na quantidade de ciclos e principalmente temperatura empregada, são discrepantes.

O uso de ciclagem mecânica foi desenvolvido objetivando a simulação da mastigação (MELLO *et al.*, 1997). Vários estudos têm mostrado sua efetividade na produção de tensão sobre restaurações, entretando, outros trabalhos demonstram não haver nenhuma influência sobre os resultados (ABDALLA & DAVIDSON, 1996; DAVIDSON & ABDALLA, 1994; DELONG & DOUGLAS, 1983; HAKIMEH *et al.*, 2000; HTANG, OHSAWA, MATSUMOTO, 2000; LUNDIN & NOREN, 1991; MUNKSGAARD & IRIE, 1988; YAP, 1997; LEIBROCK *et al.*, 1999; MCCAGHREN *et al.*, 1990; WILLIAMSON, MITCHELL, BREEDING, 1993). Assim como para ciclagem térmica, existe grande variação na metodologia empregada para uso de ciclagem mecânica. Diferenças como tipo de simulador a ser usado (simulação unidirecional, bidirecional e tridirecional), quantidade de ciclos, e força utilizada; dificultam uma comparação direta entre os diferentes estudos.

Em vista disto, o presente trabalho teve como objetivo avaliar o efeito da ciclagem térmica e mecânica em diferentes testes para avaliação de materiais que promovem adesão. A metodologia de microinfiltração tem sido extensamente empregada em estudos laboratoriais, onde variações no tipo de solução corante, tempo de imersão em solução corante e quantificação e/ou qualificação da penetração de corante não são padronizados. Recentemente, Sano *et al.*, 1994 desenvolveu outra metodologia de avaliação de infiltração, denominada nanoinfiltração. Nesta, o uso de nitrato de prata evidencia a presença de partículas de prata na base da camada híbrida, sem a presença de fendas marginais. Recentemente a detecção de partículas de nitrato de prata no corpo e topo da camada híbrida, bem como na camada adesiva foi relatado (LI & BURROW, 2000; TAY, PASHLEY, YOSHIYAMA, 2002).

A avaliação da resistência de união pode ser realizada basicamente de três maneiras: através de teste de tração, cisalhamento e microtração. O teste de cisalhamento tem sido extensivamente utilizado em estudos laboratoriais, mas apresenta como limitação a necessidade de superfícies planas de substrato dentinários, esmalte dental ou materiais. Esta forma de mensuração da resistência de união é a mais prevalente na literatura (AL-SALEHI & BURKE , 1997); no entanto, a distribuição de estresse é complexo contribuindo para a não uniformidade dos resultados entre diferentes estudos (AL-SALEHI & BURKE , 1997).

O teste de resistência a microtração foi recentemente introduzido por Sano *et al.* (1995). Esta metodologia de avaliação baseia-se no uso de pequenas áreas adesivas (0,5 – 1,0 mm^2), permitindo a avaliação de superfícies irregulares e cavidades, que são áreas clinicamente mais relevantes do que superfícies planas, comumente utilizadas para avaliação de resistência a tração e ao cisalhamento. O benefício de avaliar pequenas áreas adesivas como, por exemplo, paredes cervical, gengival e axial, assim como parede pulpar de restaurações Classe I, II, e IV, é enorme, e próximo da condição clínica em que o material vai ser utilizado; levando em consideração fatores inerentes a estas restaurações como, por exemplo, o Fator C presente em preparos cavitários (FEILZER, DE GEE, DAVIDSON, 1987).

Para este estudo os testes de cisalhamento, microinfiltraçao, microtração e nanoinfiltração foram utilizados para avaliar possíveis efeitos das ciclagens térmica e mecânica.

Para a avaliação da microinfiltração, utilizando azul de metileno, nenhum efeito da ciclagem térmica e ciclagem mecânica, bem como a associação de ambas, foi observado. Para avaliação da resistência ao cisalhamento, em superfícies planas de dentina, nenhum efeito dos diferentes tipos de estresse (térmico e mecânico) foi observado. No entanto, para as avaliações de nanoinfiltração e de microtração, um significante aumento da extensão de penetração do nitrato de prata e diminuição dos valores de adesão, respectivamente, foram observados quando espécimes foram submetidos a ciclagem térmica e mecânica.

Diferentes resultados foram encontrados de acordo com o teste a ser avaliado. Nao é possível correlacionar diretamente os resultados entre os diferentes testes pois eles foram conduzidos separadamente. Além disto, cada teste apresenta diferentes características e são conduzidos de forma distinta, mesmo tendo como objetivo comum a avaliação da resistência de união ou de infiltração.

Houve também uma variação na metodologia de ciclagem mecânica. As avaliações de resistência ao cisalhamento em superfícies planas e a avaliação de microinfiltração foram inicialmente conduzidos. Para ambos, uma quantidade de 50.000 ciclos e carga de 80N foram utlizados. Ao conduzir os testes de resistência a microtração e avaliação de nanoinfiltração optou-se pelo uso de 100.000 ciclos mecânicos e carga de 50N, com o objetivo de submeter a uma maior quantidade de ciclos e com menor carga.

A avaliação de longa duração da resistência de união foi também estudada. Foi observado que a estocagem em água por um período de 1 ano resultou na diminuição dos valores de resistência a microtração. No entanto, quando ciclagem mecânica e térmica foram utilizadas, os resultados iniciais foram significantemente menores comparados ao grupo controle e nao diminuíram significantemente após estocagem. Demonstrando que o uso da ciclagem térmica/mecânica foi capaz de reduzir os valores de adesão semelhantemente ao que a estocagem por um ano promoveu; mas, ao mesmo tempo, não promoveu efeito adicional nos valores de adesão após 1 ano de estocagem.

Desta maneira, é importante a avaliação de sistemas restauradores através de diferentes metodologias que possam proporcionar uma maior abrangência de fatores a serem considerados; e portanto maior entendimento do comportamento de cada material. O presente trabalho mostrou que a inclusão de ciclagem térmica e ciclagem mecânica associados são importantes formas de induzir tensão em materiais restauradores, e uma opção de envelhecimento das restaurações e possível simulação de condições intra-orais.

Futuros estudos devem ser desenvolvidos com o objetivo de avaliar diferentes quantidades de ciclos térmicos e mecânicos, como forma de esclarecer diferenças nos resultados apresentados desta pesquisa; possibilitando futuramente, correlacionar com idade da restauração. Também a avaliação de diferentes técnicas restauradoras e materiais restauradores devem ser avaliados.

De acordo com os estudos desenvolvidos, pode-se concluir que:

1- Para a avaliação da microinfiltração, utilizando azul de metileno, nenhum efeito da ciclagem térmica e ciclagem mecânica, bem como a associação de ambas, foi observado. Para a avaliação da resistência ao cisalhamento, nenhum efeito dos diferentes tipos de estresses (térmico e mecânico) foi observado.

2- Para a avaliação de nanoinfiltração, a associação de ciclagem térmica e mecânica resultou em significante aumento na extensão de penetração de partículas de nitrato de prata.

3- Para a avaliação de resistência a microtração, somente a associação de ciclagem térmica e mecânica resultou em significante diminuição nos valores de união.

4- Diminuição dos valores de resistência a microtração foi observada após um ano de estocagem, onde estas diferenças não foram relacionadas ao uso concomitante de ciclagem térmica e mecânica.

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