Effect of Thermal and Mechanical Load Cycling on Nanoleakage of Class II Restorations

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

Materials 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 (2000 cycles, 5 to 55°C); G3, mechanical load cycling (100,000 cycles, 50 N); G4, thermal and mechanical load cycling group (2000 cycles 5 to 5°C/100,000 cycles, 50 N). The specimens were then sealed leaving a 1 mm window around the cervical margin interface. Samples were immersed in a 50% w/v ammoniacal silver nitrate solution for 24 h, and exposed to a photodeveloping solution for 8 h. Specimens were sectioned longitudinally, embedded in epoxy resin, polished and mounted on stubs, gold sputter coated, and examined under 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 with ANOVA and Fisher's PLSD test ($p < 0.05$).

Results: The degree of nanoleakage significantly increased when thermal and mechanical cycling was performed on the same specimens, as 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.

Key words: thermal cycling, mechanical load cycling, nanoleakage, dentin, Class II restoration.

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The major goal of restorative treatment is the effective replacement of tooth structure. In order to prevent deterioration of the seal between restorative material and tooth structure, the interface must resist dimensional changes.[21] Even after controlling the effects of polymerization shrinkage, deterioration of the restoration may subsequently occur due to chemical, thermal, and mechanical load stresses.[1,2,16,21] 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.[2,11,17,21,28]

The use of thermal cycling is frequently included in laboratory studies evaluating microleakage[3,10,15,29] and more recently in nanoleakage evaluation.[18] The effectiveness of this method in altering the restoration interface has, however, been questioned.[3,15] Several studies sug-

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gest that occlusal mechanical cycling could accelerate the deterioration of the dentin/restoration interface.[1,2,10,21] Sealing ability evaluation[1,2,11,12,17,19,21,28,29] has included mechanical load cycling in experimental protocols.

The sealing ability of different materials can be evaluated using microleakage tests and, more recently, nanoleakage tests. The term "nanoleakage" has been used to describe microporous zones as the pathway for degradation of a bonded interface, either in incompletely cured adhesive resin, within the hybrid layer, and/or demineralized dentin, that allow tracer penetration to occur in the absence of interfacial gaps.[30] The advantage of the nanoleakage test as compared to the microleakage test is that failure of an optimal seal can be detected without the necessity of gap formation, while microleakage testing detects leakage only in the presence of gaps. Nanoleakage testing can also be used to evaluate areas of a bonded interface that are not necessarily at a restoration margin.[30]

Since limited information exists on the effect of different oral stresses on the 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

Specimen Preparation

Forty bovine incisors were selected, cleaned of debris with curettes and pumice paste at low speed, and stored in a 0.1% sodium azide saline solution. Incisal surfaces were cut 4 mm above the cemento-enamel junction under water refrigeration.

Slot preparations were made 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.[6] 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, St Paul, MN, USA) and composite resin Z-250 (3M-ESPE). The adhesive system was applied according manufacturers' instructions: acid etch for 15 s, rinse for 15 s, blot dry, apply two consecutive coats of the adhesive, lightly air dry, and light cure for 10 s. The preparation was filled with the microhybrid composite resin Z-250 in two horizontal increments and light cured for 40 s 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 was measured periodically by a radiometer (Optilux, Demetron/Kerr, Orange, CA, USA) and 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 h. Afterwards, they were finished and polished with Al_2O_3 abrasive disks (Sof-Lex Pop-on, 3M-ESPE). The teeth were then randomly divided into 4 groups:

G1 = control group (no thermal or mechanical load cycling)
G2 = thermal cycling only (2000 cycles, 5 to 55°C)
G3 = mechanical load cycling only (100,000 cycles/load at 50 N)
G4 = thermal cycling (2000 cycles, 5 to 55°C) and mechanical cycling (100,000 cycles/load at 50 N)

Specimens from all groups were kept in distilled and deionized water until immersion in dye solution. The roots of specimens subjected to mechanical load cycling were partly embedded in epoxy resin (Buehler, Lake Bluff, IL, USA) 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 2000 cycles in a thermocycling apparatus (MCT2, AMM 2 Instrumental, Sao Paulo, SP, Brazil) with baths of 5 ± 2°C and 55 ± 2°C, a dwell time of 60 s, and a transfer time of 7 s 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-made by Dentsply/Caulk Technical Research, Milford, DE, USA), modified for loading testing. The apparatus consisted of 4 stainless steel pistons, to the ends of which a polyacetal cylinder tip (15 mm diameter) was attached (Fig 1). The pistons performed only axial movement, without the additional 30-degree rotation usually employed for wear evaluation.[7] The loading device delivered an intermittent axial force of 50 N at 2 cycles/s for a total of 100,000 cycles. In group G4, thermal cycling was performed first and mechanical cycling after the thermal cycling was completed, due to the impossibility of conducting both simultaneously. During all mechanical load cycling procedures, specimens were kept immersed in distilled and deionized water.

Immersion in Dye and Nanoleakage Evaluation

After thermocycling and mechanical load cycling, the apices and occlusal portions of all specimens from all groups 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 rim around the cervical margin.[30] The teeth were immersed in a 50% ammoniacal silver nitrate solution for 24 h.[33] The tracer solution was prepared by dissolving 25 g of silver nitrate crystals (Sigma Chemical, St Louis, MO, USA) in 25 ml of distilled water. Concentrated (28%) ammonium hydroxide (Sigma Chemical) 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 photodeveloping solution for 8 h under a fluorescent light.

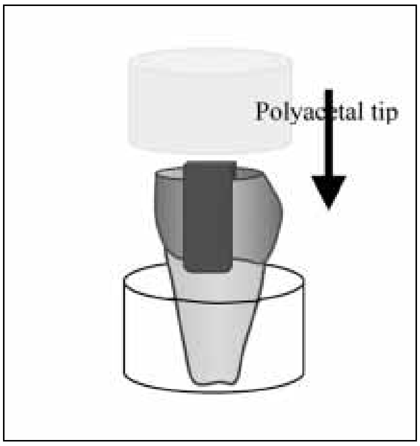

Fig 1 Illustration of load application on the occlusal surface. Black arrow indicates direction of loading force.

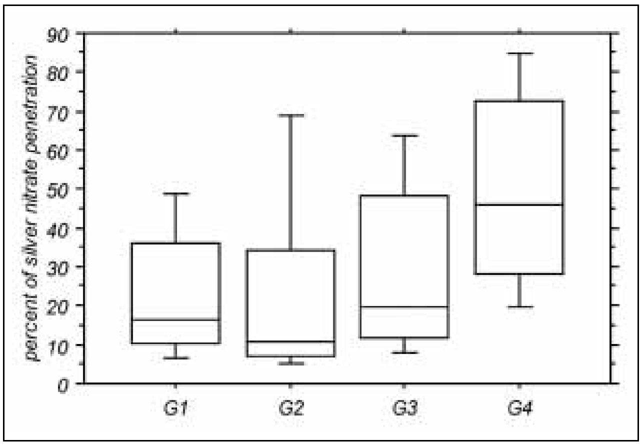

Fig 2 Box plot of nanoleakage data distribution.

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

A total of 20 sections was obtained for each group. All the sections were embedded in epoxy resin (Buehler) and polished with silicon carbide papers of ascending grits (600, 800, 1200; Buehler) and diamond pastes (6, 3, 1 µm; Buehler). Specimens were then ultrasonically cleaned, air dried, mounted on stubs, left to rest for 24 h, gold sputter coated (Polaron E-5200 Energy Beam Sciences, Agawan, MA, USA), and examined in a scanning electron microscope (model 6500, JEOL, Peabody, MA, USA) using backscattered electron and second beam modes. EDX analysis was performed randomly to exclude false positive detection of silver particles by reflection.[26]

The length of silver nitrate penetration along the cervical wall was measured using different magnifications (200X to 5000X), permitting precise localization of the silver nitrate trace. The length of silver nitrate penetration was quantitatively measured using a caliper directly on the SEM screen. Measurements started at the outermost surface of the restoration, extending towards the axial wall. The leakage from the gingival margin was calculated as the percentage of the total length of cut dentin surface penetrated by silver nitrate, ie, the ratio of the length of silver nitrate penetration along the cervical dentin/resin interface to the total length of the cervical restoration wall. Specimens evaluated were marginal-gap free. Statistical analysis was performed using ANOVA and Fisher's PLSD test ($p < 0.05$).

RESULTS

The mean nanoleakage length and standard deviation for each group are described in Table 1 and data distributions are depicted in Fig 2. One-way ANOVA revealed statistically significant differences 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 cycling (G4) was performed on the same specimens as compared to the other groups (G1, G2, and G3). No differences were observed between the control, thermal cycling, and mechanical cycling groups.

Table 1 Means and standard deviation for nanoleakage values

Groups	Thermal cycling (2000 cycles/ 5–55°C)	Mechanical cycling (100,000 cycles/50 N)	Mean (SD)* (%)
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)
* Different superscript letters indicate a statistically significant difference

The SEM evaluations using backscattered mode offered more accurate visualization of the silver particles (Figs 3a and 3b) compared to that provided by the secondary electron beam mode. Therefore, all the nanoleakage analysis was done with the backscattered mode. Different nanoleakage patterns were observed: silver was present at the bottom of the hybrid layer (Fig 4), within the adhesive layer (Fig 5a), and at the bottom of the adhesive or top of the hybrid layers (Fig 5b). 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 (Fig 6).

DISCUSSION

The durability of the bond between adhesive resins and dentin is of critical importance for the longevity of bonded restorations.[25] It is common to have the margins of cavi-

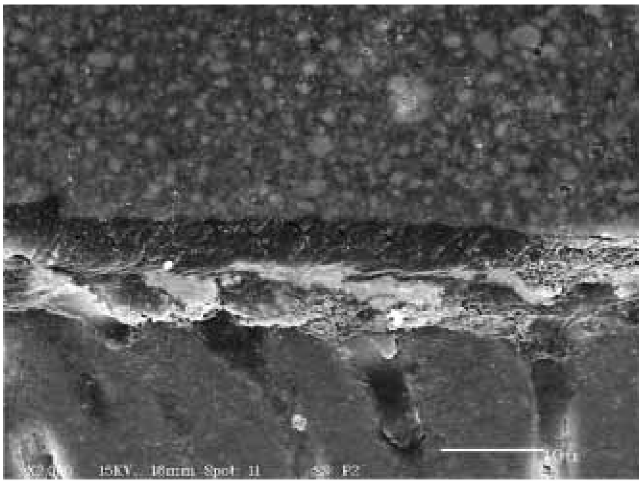

Fig 3a SEM secondary beam micrograph of the dentin/restoration interface.

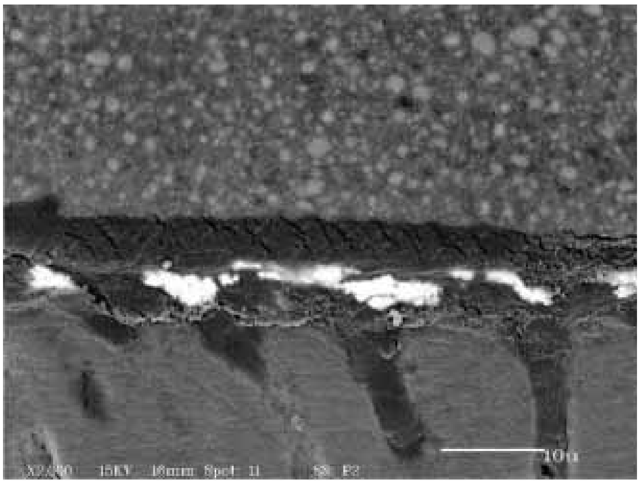

Fig 3b SEM backscattered micrograph of the dentin/restoration interface. The backscattered mode offers more accurate visualization and analysis of the silver nitrate deposition.

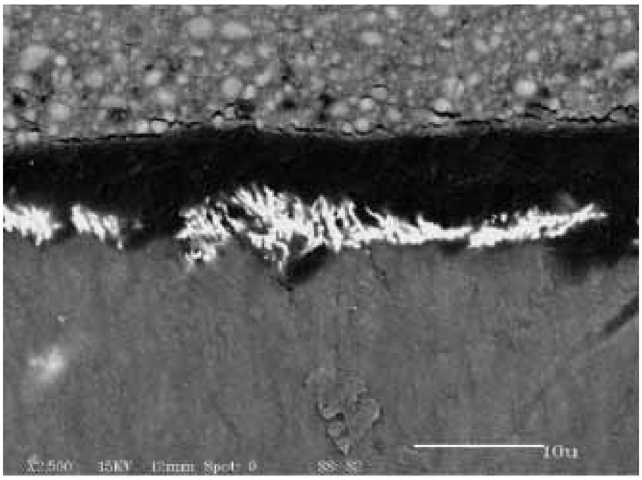

Fig 4 SEM micrograph of the interface shows silver nitrate particle deposition at the bottom of the hybrid layer.

ties in dentin due to the extensive loss of tooth structure by decay. The dentin substrate remains a challenge when trying to achieve a reliable bond and seal, due to the complex characteristics of the substrate. In this study, bovine teeth were used in place of human teeth,[23,27] 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.[1-3,11,15,17,21,28] Although the in vitro effect of thermal cycling on microleakage has been thoroughly investigated,[3,10,15,29] few studies have reported its effect on nanoleakage.[12,18] The quantity of cycles and the temperatures used seem to constitute the major difference between studies.[3,15,29] Controversial results have been reported regarding the influence of thermal cycling on microleakage[9,10,15,29] and the lack of influence on nanoleakage measurements.[12,18]

Two thousand cycles and temperatures of 5 to 55°C were chosen as an average of the number of cycles employed in different studies, and the use of ISO standardized 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 finding is in accordance with other studies, these studies used different numbers of cycles, bath temperatures, and nanoleakage tracers.[12,18] 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.[8,13]

The use of mechanical load cycling has been studied due to its potential for simulating mastication. In the present study, the use of a cylindrical polyacetal tip touching only the material was intended to fatigue the restoration. It is difficult, if not impossible, to simulate the occlusal forces, due to variations in age, gender, and type of tooth. These factors interfere with the analysis of the effects of force. However, the simulation of a mean force is necessary for further comparison between studies. The force of 50 N was chosen as a moderately low force present during mastication,[4] which, when employed for a high number of cycles (100,000 in this study), can create a continuous stroke aimed to fatigue primarily the restoration interface.

For microleakage evaluation, discrepant results related to the effect of load cycling have been reported.[1,5,15,21,29] In the present study, no statistically significant influence was observed when only mechanical load cycling was performed. Li et al[19] found no effect of load cycling on nanoleakage on either flat surfaces or Class V restorations. Although their results are in accordance with those of the present study, Class II restorations were examined here, meaning the C-factors[13] and consequently polymerization shrinkage are different, and could have some influence on the results.

In the present study, neither mechanical nor thermal cycling had any effect on nanoleakage values when

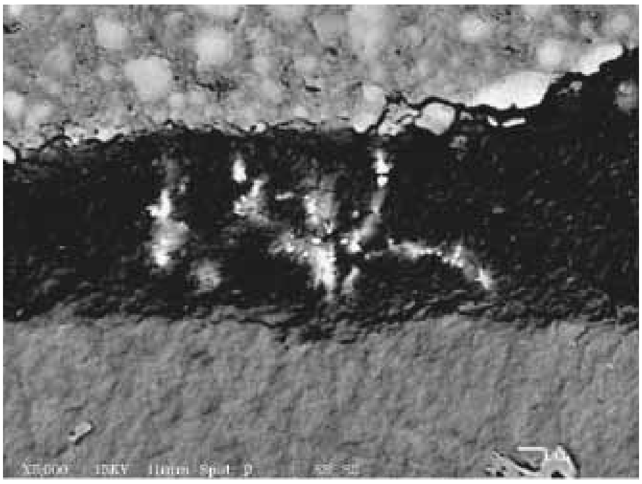

Fig 5a SEM micrograph of the interface shows silver nitrate particle deposition at the adhesive layer and hybrid layer. Interface cracks are SEM processing artifacts.

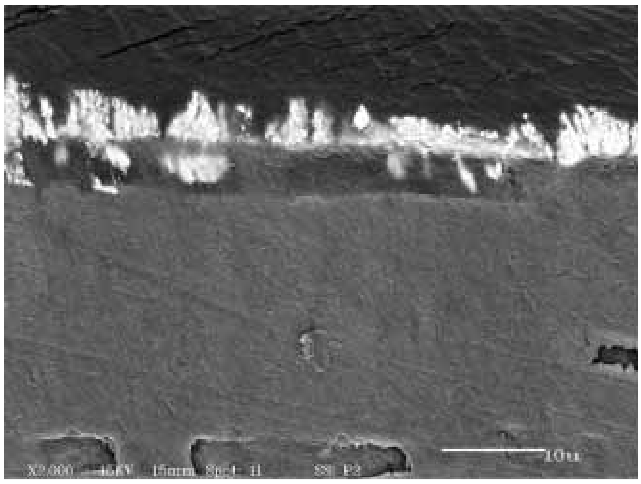

Fig 5b SEM micrograph of the interface showing silver nitrate particle deposition at the body and top of the hybrid layer.

applied alone; however, the association of the two significantly increased the length of silver nitrate penetration. Nara et al[24] observed that the association of thermal and load cycling was effective to examine microleakage of cervical composite restorations. Bedran-de-Castro et al,[7] evaluating microtensile bond strength on cervical walls of Class II cavity preparations performed under the same conditions and with the same group divisions as the present study, found a decrease in bond strength values when thermal and mechanical load cycling were performed on the same specimens. According to their study, the use of mechanical load cycling resulted in a change of fracture mode 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[30] and between the bottom of the adhesive and the top of the hybrid layers.[20] The ammoniacal silver nitrate solution[33] was used in the present study to avoid the acidity present in the conventional silver nitrate solution, which could result in artifacts such as tracer penetration due to demineralization of unprotected dentin. Using this silver nitrate modified solution, Tay et al[33] 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.[33] These phenomena have been observed for self-etching[33] and total etching adhesive systems.[32] Tay et al[33] 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.[34] In addition, the use of a moist bonding technique in cavity preparations makes

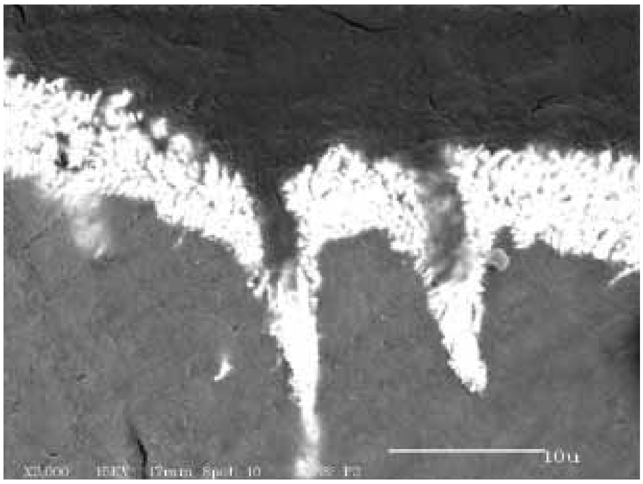

Fig 6 SEM micrograph shows high deposition of silver particles at the bottom of the hybrid layer, observed for mechanical load cycling specimens.

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.[33] We suggest that the presence of water voids at the adhesive layer and/or interface layer[22] 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 between 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. Given our data, we cannot fully elucidate 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 the restorations examined were small. This effect on bonding may contribute to the effectiveness of mechanical load cycling in fatiguing the restoration through the weakest points present (ie, water and adhesive voids, unprotected collagen present in the demineralized dentin zone, and water treeing).

Degradation of the adhesive interface due to mechanical loading is a very important issue in restorative dentistry, since patient demand for posterior composite restorations has significantly increased. A similar nanoleakage pattern was observed for restorations prepared in vivo and in vitro.[14] The use of nanoleakage testing methods intends to identify pathway of leakage within the hybrid layer.[30] The hybrid layer is a major link for bonding between composite and dentin; thus, alterations in this hybrid layer may be directly related to the durability of the restoration.[31]

Further studies should be conducted to evaluate 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 testing methods.

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. Thermal and mechanical cycling combined adversely affected nanoleakage values. Accurate simulation of the oral conditions might be crucial to better evaluate and understand the performance of adhesive materials in laboratory tests.

ACKNOWLEDGMENTS

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REFERENCES

1. Abdalla AI, Davidson CL. Comparison of the marginal integrity of in vivo and in vitro class II composite restorations. J Dent 1993;21:158-162.
2. Abdalla AI, Davidson CL. Effect of mechanical load cycling on the marginal integrity of adhesive class I resin composite restorations. J Dent 1996;24:87-90.
3. Alani AH, Toh CG. Detection of microleakage around dental restorations: a review. Oper Dent 1997;22:173-185.
4. Anderson DJ. Measurement of stress in mastication. I. J Dent Res 1956;35:664-670.
5. Ausiello P, Davidson CL, Cascone P, de Gee A, Rengo S. Debonding of adhesively restored deep class II MOD restorations after functional loading. Am J Dent 1999;12:84-88.
6. Bedran-de-Castro AK, Pimenta LA, Amaral CM, Ambrosano GM. Evaluation of microleakage in cervical margins of various posterior restorative systems. J Esthet Restor Dent 2002;14:107-114.
7. Bedran-de-Castro AK, Pereira PNR, Pimenta LAF, Thompson JY. Effect of thermal and mechanical load cycling on microtensile bond strength of a total-etch adhesive system. Oper Dent 2004;29:150-156.
8. Bouillaguet S, Ciucchi B, Jacoby T, Wataha JC, Pashley DH. Bonding characteristics to dentin wall of class II cavities, in vitro. Dent Mater 2001;17:316-321.
9. Chan MF, Glyn-Jones JC. Significance of thermal cycling in microleakage analysis of root restorations. J Dent 1994;22:292-295.
10. Darbyshire PA, Messer LB, Douglas WH. Microleakage in class II composite restorations bonded to dentin using thermal and load cycling. J Dent Res 1988;67:585-587.
11. DeLong R, Douglas WH. Development of an artificial oral environment for the testing of dental restoratives: bi-axial force and movement control. J Dent Res 1983;62:32-36.
12. Dörfer CE, Staehle HJ, Wurst MW, Duschner H, Pioch T. The nanoleakage phenomenon: influence of different dentin bonding agents, thermocycling and etching time. Eur J Oral Sci 2000;108:346-351.
13. Feilzer AJ, de Gee AJ, Davidson CL. Setting stress in composite resin in relation to configuration of the restoration. J Dent Res 1987;66:1636-1639.
14. Ferrari M, Tay FR. Technique sensitivity in bonding to vital, acid-etched dentin. Oper Dent 2003;28:3-8.
15. Hakimeh S, Vaidyanathan J, Houpt ML, Vaidyanathan TK, Hahen SV. Microleakage of compomer class V restorations: effect of load cycling, thermal cycling, and cavity shape differences. J Prosthet Dent 2000:83:194-203.
16. Jørgensen KD, Itoh K, Munksgaard EC, Asmussen E. Composite wall-to-wall polymerization contraction in dentin cavities treated with various bonding agents. Scand J Dent Res 1985;93:276-279.
17. Krejci I, Reich T, Lutz F, Albertoni M. In-vitro Testverfahren zur Evaluation dentaler Restaurationssysteme. Schweiz Monatsschr Zahnmed 1987;100:953-959.
18. Li H, Burrow MF, Tyas MJ. The effect of thermocycling regimens on the nanoleakage of dentin bonding systems. Dent Mater 2002;18:189-196.
19. Li H, Burrow MF, Tyas MJ. The effect of load cycling on the nanoleakage of dentin bonding systems. Dent Mater 2002;18:111-119.
20. Li H, Burrow MF, Tyas MJ. Nanoleakage patterns of four dentin bonding systems. Dent Mater 2000;16:48-56.
21. Mello FSC, Feilzer AJ, de Gee AJ, Davidson CL. Sealing ability of eight resin bonding systems in a class II restoration after mechanical fatiguing. Dent Mater 1997;13:372.
22. Nakabayashi N, Pashley DH. Evolution of dentin resin bonding. In: Hybridization of Dental Hard Tissues. Tokyo: Quintessence, 1998:1-17.
23. Nakamichi I, Iwaku M, Fusayama T. Bovine teeth as possible substitutes in the adhesion test. J Dent Res 1983;62:1076-1081.
24. Nara Y, Suzuki T, Kizuki I, Miyamoto M, Kimishima T, Maseki T, Tanaka H, Dogon IL. Effect of thermal cycling and/or repeated load on microleakage of cervical composite restoration [abstract 3387]. J Dent Res 2002;81:A-418.
25. Okuda M, Pereira PN, Nakajima M, Tagami J. Relationship between nanoleakage and long-term durability of dentin bonds. Oper Dent 2001;26:482-490.
26. Pioch T, Staehle HJ, Duschner H, Garcia-Godoy F. Nanoleakage at the composite-dentin interface: a review. Am J Dent 2001;14:252-258.
27. Reeves GW, Fitchie JG, Hembree JH, Puckett AD. Microleakage of new dentin bonding systems using human and bovine teeth. Oper Dent 1995;20:230-235.
28. Roulet JF. Degradation of dental polymers. Karger: Basel, 1990:108-110.
29. Prati C, Tao M, Simpson M, Pashley DH. Permeability and microleakage of class II resin composite restorations. J Dent 1994;22:49-56.
30. Sano H, Takatsu T, Ciucchi B, Horner JA, Matthews WG, Pashley DH. Nanoleakage: leakage within the hybrid layer. Oper Dent 1995;20:18-25.
31. Spencer P, Swafford JR. Unprotected protein at the dentin-adhesive interface. Quintessence Int 1999;30:501-507.
32. Tay FR, Pashley DH. Water treeing – a potential mechanism for degradation of dentin adhesives. Am J Dent 2003;16:6-12.
33. Tay FR, Pashley DH, Yoshiyama M. Two modes of nanoleakage expression in single-step adhesives. J Dent Res 2002;81:472-476.
34. Zheng L, Pereira PNR, Nakagima M, Sano H, Tagami J. Relationship between adhesive thickness and microtensile bond strength. Oper Dent 2001;26:97-104.

Clinical relevance: Laboratory simulation of oral conditions, ie, thermal/mechanical stresses, may be important to predict the clinical performance of adhesive materials.