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Effect of Restorative System and Thermal Cycling on the Tooth-Restoration Interface – OCT Evaluation

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Clinical Relevance

The self-etching adhesive system (CSE) showed better dentin marginal integrity after thermal cycling, compared with the etch-and-rinse (SB2), regardless of the type of resin composite used. Enamel was not affected even after thermal cycling.

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SUMMARY

The present study evaluated the tooth/noncarious cervical lesion restoration interface when using different adhesive systems and resin composites, submitted to thermal cycling (TC), using optical coherence tomography (OCT). Noncarious cervical lesion (NCCL) preparations (0.7 mm depth × 2 mm diameter) were performed on 60 human third molars and randomly divided into six groups, according to the adhesive system and resin composite used: group 1 = Adper Single Bond 2 (SB2) + Aelite LS Posterior (AP); group 2 = SB2 + Venus Diamond (VD); group = SB2 + Filtek Z250XT (Z250); group 4 = Clearfil SE Bond (CSE) + AP; group 5 = CSE + VD; group 6 = CSE + Z250. Selective enamel etching was performed for 30 seconds on groups 4, 5, and 6, while groups 1, 2, and 3 were etched for 30 seconds in enamel and 15 seconds in dentin. All groups were evaluated using OCT before and after TC (n=10). Images were analyzed using Image J software; enamel and dentin margins were separately

evaluated. Data from OCT were submitted to PROC MIXED for repeated measurements and Tukey Kramer test ($\alpha = 0.05$). No marginal gaps were observed in etched enamel, either before or after TC, for all adhesive and resin composite systems. A significant interaction was found between adhesive system and TC for the dentin groups; after TC, restorations with CSE showed smaller gaps at the dentin/restoration interface compared with SB2 for all resin composites. Increased gap percentages were noticed after TC compared with the gaps before TC for all groups. In conclusion, TC affected marginal integrity only in dentin margins, whereas etched enamel margins remained stable even after TC. Dentin margins restored with CSE adhesive system showed better marginal adaptation than those restored with SB2. Resin composites did not influence marginal integrity of NCCL restorations.

INTRODUCTION

Clinical success of resin composite restorations is fundamentally dependent on effective and durable bonds to enamel and dentin.[1] Marginal sealing is one of the most important factors influencing the success of a restoration.[2]

Although an intimate bond is extremely important, a perfect margin is difficult to achieve[3] because of intrinsic characteristics of the materials. Gaps can occur in enamel and dentin because of loss of internal adaptation among dental hard tissues and the resin composite material. Additionally, bonding to dentin is the most difficult type of bond to achieve.[4]

With regards to clinical success, one of the most important factors to be considered is composite polymerization shrinkage.[5] Over the past few years, manufacturers have invested in the development of low-shrinkage resin composites. They claim that such materials produce a lower percentage of shrinkage compared with conventional composites, which would be extremely useful in improving the marginal adaptation of restorations.[6] Shrinkage from polymerization can result in marginal gaps and leakage, tooth fracture, composite fracture, dislodgement of the restoration, and postoperative sensitivity.[2] Thus, the use of a resin composite with an appropriate elastic modulus and low rate of polymerization shrinkage, combined with an adequate dentin adhesive system, could be an effective way to restore cervical lesions.[7]

Because of the unique characteristics of noncarious cervical lesions (NCCLs)—such as a sharp wedge-like morphology; a frequently subgingival location,[8] which includes enamel and dentin margins; and the need for supporting occlusal and brushing forces—the adhesion of materials to these lesions becomes a challenge. NCCL Class V cavities are frequently used to clinically evaluate the effectiveness of adhesive systems.[9] High adhesion levels are necessary to fulfill such tasks as sealing dentinal tubules to reduce postoperative sensitivity, sealing restoration margins to reduce the risk of marginal staining and marginal caries, and keeping the restoration in place.[9] In the case of retention, while studies with class I and II cavities are of great value[10,11] when evaluating a restoration, the preparation and/or caries removal normally generates adequate mechanical retention, thus making adhesion to tooth substance less important.[9] Adhesion to tooth substrate is more necessary when there is not sufficient retention, as with NCCL restorations.[9]

Regarding the conventional bonding technique, etching dentin is an aggressive procedure, as it dissolves and removes the natural collagen protection, thereby producing a resin-collagen complex that is vulnerable to degradation by water sorption, which is possibly enhanced by the documented enzymatic degradation process.[12] The advent of two-step self-etching adhesive systems introduced a new perspective, as these materials limit dentin mineral dissolution while simultaneously replacing minerals with resin monomers.[13] Although self-etching adhesive systems exhibit the best dentin marginal quality, they frequently do not show the same superiority in enamel, as demonstrated by Frankenberger and others.[14] When using this type of adhesive, prior selective enamel etching is commonly indicated to promote a superior demineralization and micromechanical retention.[14,15] Even so, the strongest chemical bonds can be weakened when subjected to repeated disruptive stresses in the oral environment.[8]

In vitro studies can be performed to simulate oral environment stresses. Thermal cycling (TC) simulates temperature changes in the oral environment.[16] TC effects are deleterious to the tooth/restoration bonding interface and can accelerate exposure of resin components to hydrolytic degradation or significant temperature oscillations, which may cause tensions in the bonding interface.[17]

Evaluation of the tooth/restoration interface may be performed by various methods. In an attempt to elucidate problems related to destructive analyses,

Table 1: *Composition, Manufacturers, Batch Numbers, and Protocol for Applying the Materials Studied*

Material (Group)	Manufacturer (Batch no.)	Composition	Application Protocol
Clearfil SE Bond (CSE)	Kuraray Inc, Osaka, Japan (Primer: 01108A; Bond: 01657A)	Primer: HEMA (10-30 wt%), MDP, hydrophilic aliphatic dimethacrylate, dl-camphorquinone, water, accelerators, eyes, others Bond: BISGMA (25-45 wt%), HEMA (20-40 wt%), MDP, hydrophobic aliphatic methacrylate, colloidal silica, dl-camphorquinone, initiators, accelerators, others	Etch with 35% phosphoric acid on enamel for 30 s. Rinse for 30 s. Blot excess water using a humid cotton pellet. Immediately after blotting, apply a coat of the primer with gentle agitation and using a fully saturated applicator for 20 s. Gently air thin and apply a coat of the bond. Gently air thin for 5 s to evaporate solvent. Photoactivate for 20 s.
Adper Single Bond 2 (SB2)	3M ESPE Dental Products, Sumaré, Brazil (N3025G0BR)	Ethyl alcohol (25-30 wt%), silane-treated sílica (nanofiller) (10-20 wt%), BISGMA (10-20 wt%), HEMA (5-15 wt%), glycerol 1,3-dimethacrylate (5-10 wt%), copolymer of acrylic and itaconic acids (5-10 wt%), UDMA (1-5 wt%), water (<5 wt%)	Etch with 35% phosphoric acid, applied first to enamel (30 s) and then to dentin (15 s). Rinse for 30 s. Blot excess water using a humid cotton pellet. Immediately after blotting, apply a coat of adhesive with gentle agitation using a fully saturated applicator for 20 s. Repeat the application. Gently air thin for 5 s to evaporate solvent. Photoactivate for 20 s.
Aelite LS Posterior (AP)	Bisco, Schaumburg, IL, USA (111200007310)	Bis-EMA (<25 wt%), glass filler (<65 wt%), amorphous silica (<15 wt%)	Place in a single increment; photoactivate for 20 s
Venus Diamond (VD)	Heraeus Kulzer Inc, Hanau, Germany (010039)	TCD-DI-HEA, UDMA, barium aluminum fluoride glass, highly discrete nanoparticles (contains 64% filler by volume, 5 nm-20 μm)	Place in a single increment; photoactivate for 20 s
Filtek Z250 XT (Z250)	3M ESPE, Sumaré, Brazil (N333058BR)	Silane-treated ceramic (65-90 wt%), BISGMA (1-10 wt%), Bis-EMA (1-10 wt%), silane-treated silica (1-10 wt%), UDMA (1-10 wt%)	Place in a single increment; photoactivate for 20 s

nondestructive methods used to evaluate marginal integrity of restorations have been studied. Recently, there has been an increasing interest in technologies that can reconstruct images of the internal structures to study defects of resin composites, adhesion, and shrinkage phenomena with a minimum of technique artifacts.[18] Optical coherence tomography (OCT) is an emerging technology that has demonstrated its utility in assessing and visualizing internal biological structures and some biomaterials in a noninvasive and nondestructive manner.[10,11,18-21] OCT has been used to identify and quantify marginal gaps under a resin composite restoration without specimen cross-sectioning.[10,11,18,19,21] In a recent study, Bakhsh and others,[11] showed that OCT images of some cavities provided increased signal intensity that appeared as bright clusters at the cavity floor and represented a gap at the bonded interface.

Thus, the aim of this study was to use OCT to quantitatively evaluate how different adhesive systems associated with resin composites of different shrinkage rates as well as TC affect NCCL marginal integrity (enamel/dentin). The following hypotheses were tested: 1) TC affects marginal integrity of the enamel-dentin/restoration interfaces; 2) the self-etching adhesive system produces a lower marginal gap percentage in enamel and dentin compared with the etch-and-rinse adhesive system; and 3) low shrinkage resin composites produce a lower marginal gap than conventional resin composites.

METHODS AND MATERIALS

Restoration Procedures

The materials, manufacturers, composition, batch number, and application protocol of the materials used in this study are indicated in Table 1.

Sixty sound, freshly extracted, human third molars were obtained according to protocols approved by the ethical committee and stored in deionized water at 4°C for up to 30 days. A standardized NCCL preparation (0.7 mm depth × 2 mm diameter) was performed in each tooth, with cavity margins located in both enamel and dentin. Preparations were created using one diamond bur (standard grain 75-125 μm, no. 3131, Microdont, São Paulo, Brazil) for every five cavities in a high-speed handpiece with a cooled water spray using a standardized cavity preparation machine.[22] The teeth were pumiced and then randomly divided into six groups (n=10) according to the adhesive system and resin composite used: group 1 = Adper Single Bond 2 (SB2) + Aelite Posterior (AP); group = SB2 + Venus Diamond (VD); group 3 = SB2 + Filtek Z250 XT (Z250); group 4 = Clearfil SE Bond (CSE) + AP; group 5 = CSE + VD; and group 6 = CSE + Z250. Bonding and restorative procedures are indicated in Table 1. Enamel etching was performed for 30 seconds before application of the adhesive systems for all groups, while dentin etching was performed for 15 seconds only for the groups with the SB2 adhesive system.[14] Composites were photo activated according to the manufacturer's recommendations, using a light-emitting diode curing light (700 mW/ cm^2 intensity; Elipar Freelight 2, 3M ESPE, St Paul, MN, USA). Teeth were then stored for 24 hours at 37°C at 100% humidity. Finishing and polishing were performed using a sequence of medium, fine, and superfine aluminum-oxide abrasive disks (Sof-Lex Pop On, 3M ESPE) for 15 seconds each.

OCT Evaluation

After preparation and polishing procedures, a silicon specimen holder (Silon 2APS, Dentsply, Catanduva, Brazil) was fabricated for each specimen to individually fix it to the OCT worktable (OCS1300SS, Thorlabs Inc, Newton, NJ, USA) and allow identical assessment of each specimen before (baseline) and after TC. Images were obtained by scanning the buccal surface in the mesiodistal direction over the restoration. Five images were obtained every 0.33 mm.

Marginal Gap Percentage Calculation

Images were quantitatively analyzed using Image J software (Image J 1.45, NIH, Bethesda, USA).[23]

Enamel Marginal Gap Percentage Calculation

First, total enamel marginal length was calculated. The enamel marginal gap was linearly measured along the enamel margin. Then, a percentage of enamel marginal gap was calculated using equation 1, where %G1= % enamel marginal gap at baseline; L_e= total enamel marginal length; and l_e= enamel marginal gap length:

Equation 1: %G1 = (le / Le) *100

After baseline evaluation, specimens were thermal cycled for 1000 cycles (30 seconds in each bath of 5°C and 55°C water, with an interval of 30 seconds in a 37°C bath) in a thermal cycling simulator machine (MSCT-3, Elquip, São Carlos, Brazil). After TC, each specimen was fixed on the holder and the gap evaluation was carried out again, using the same parameters and locations as the baseline, to obtain %G2:

Equation 2: %G2 = (le / Le) *100

The enamel marginal gap was calculated as follows: $\%Gap = \%G2 - \%G1$, where %G1 = the pre-TC enamel marginal gap and %G2 = the post-TC enamel marginal gap.

The same procedures for enamel marginal gap measurement were conducted to measure dentin marginal gap, with the dentin marginal gap percentage calculated as follows:

1. Dentin gap percentage at baseline: $\%G1 = (ld/Ld) \times 100$
2. Dentin gap percentage after TC: $\%G2 = (ld/Ld) \times 100$
3. Dentin gap percentage: $\%Gap = \%G2 - \%G1$

Only the image with the highest percentage of enamel and dentin marginal gaps from each group was considered for statistical analysis.

After exploratory data analysis of enamel and dentin gap percentages, as analyzed by OCT, the methodology of mixed models for repeated measures (PROC MIXED for repeated measures) and the Tukey Kramer test for comparison between groups were applied. Level of significance was set at 5%.

RESULTS

No gaps were observed at the enamel/restoration interface before or after TC for any resin composite or adhesive system used (Figures 1 through 3). When considering the dentin/restoration interface, statistical analysis showed no interaction among the three factors studied (resin composites, adhesive systems, and TC) (p=0.3557). However, there was a significant interaction between the factors of adhesive system and TC (p<0.0001) (Table 2).

Table 2 shows the results of the OCT analysis of the dentin/restoration interface, providing the mean percentage and standard deviation of the dentin gap formation based on TC, resin composite, and adhesive system. The CSE and SB2 adhesive systems showed significantly higher dentin marginal gap

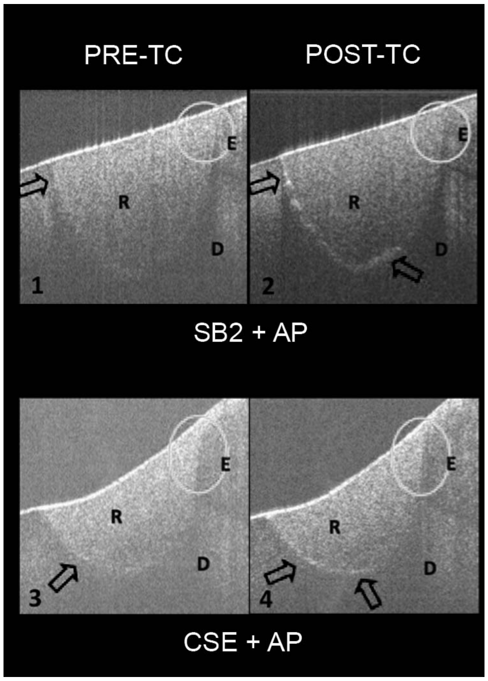

Figure 1. *OCT image from a sample restored with AP resin composite. The first column shows OCT images before TC, and the second column represents after TC. The first line shows samples restored with the SB2 adhesive system, and the second line shows samples restored with CSE. Arrows indicate gaps in the resin/dentin interface observed by OCT. Note the absence of resin/enamel interfacial gaps, even after TC, shown in circles. R, resin composite; E, enamel; D, dentin.*

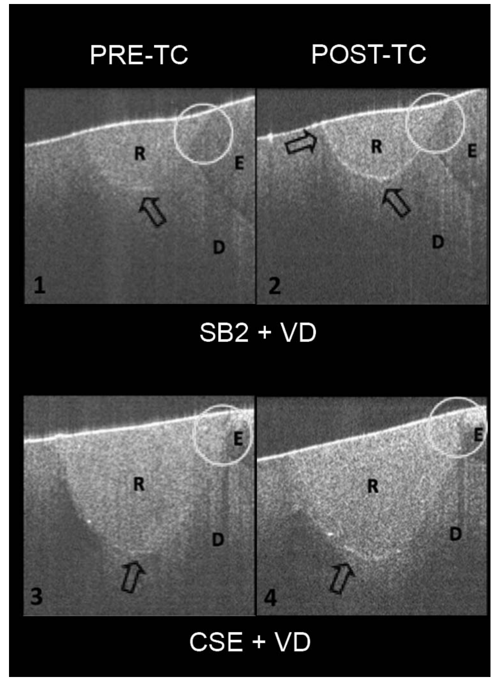

Figure 2. *OCT image from a sample restored with VD resin composite. The first column shows OCT images before TC, and the second column represents after TC. The first line shows samples restored with the SB2 adhesive system, and the second line shows samples restored with CSE. Arrows indicate gaps in the resin/dentin interface observed by OCT. Note the absence of resin/enamel interfacial gaps, even after TC, shown in circles. R, resin composite; E, enamel; D, dentin.*

means at the resin/dentin interface after TC than the same groups at baseline.

When analyzing the effect of the adhesive systems after TC, restorations using SB2 showed a higher mean percentage of dentin marginal gaps, compared with those obtained with CSE, for all tested resin composites. No significant difference in marginal gaps was found between measures obtained before and after TC for all resin composites used. Figures 1 through 3 show OCT images before and after TC for each resin composite and adhesive system in the enamel and dentin margins.

DISCUSSION

The first hypothesis was partially accepted because TC did not affect enamel/restoration margins but did affect dentin/restoration margins.

An absence of enamel marginal gaps was observed in all groups, even after TC. These results were different from those obtained by Makishi and others,[10] who used 5000 cycles. Those authors observed an increase in enamel gap formation after TC. The difference in these results demonstrates that long-term TC can present differences in the bonding of different adhesive systems to enamel. Although ISO TR 11450[24] recommends 500 cycles as a methodology for aging studies, the current study used 1000 thermal cycles and still did not demonstrate differences between groups. In addition, this difference in results might be attributed to the enamel surface acid etching before the use of the self-etching adhesive system, which was not performed in the study by Makishi and others.[10] Although all self-etching adhesives bond reasonably well to ground enamel, there is a general consensus that the milder versions of these adhesives do not etch well on unground surfaces, where there is no resin tag formation and little subsurface demineralization for micromechanical retention.[15,25,26] Thus, we chose to do enamel etching using phosphoric acid before CSE application, as indicated by Frankenberger and others[14] It can improve the bond stability of these adhesive systems to enamel, hindering the formation of marginal gaps and producing greater longevity for the restoration.[14] This may have contributed to the absence of statistical difference between adhesive systems in enamel margins.

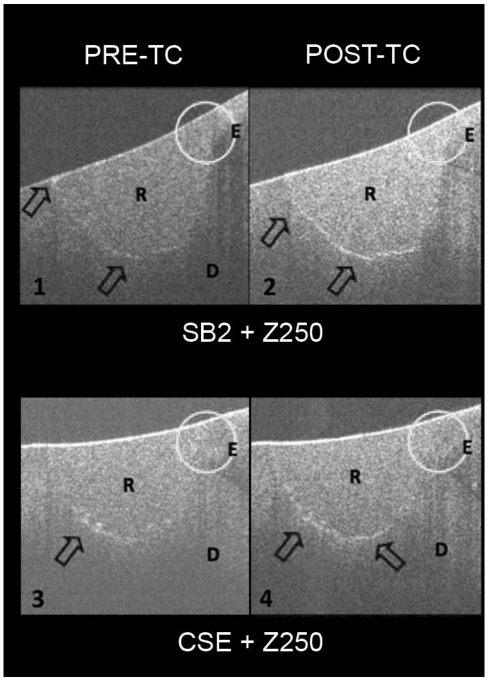

Figure 3. *OCT image from a sample restored with Z250 resin composite. The first column shows OCT images before TC, and the second column represents after TC. The first line shows samples restored with the SB2 adhesive system, and the second line shows samples restored with CSE. Arrows indicate gaps in the resin/dentin interface observed by OCT. Note the absence of resin/enamel interfacial gaps, even after TC, showed in circles. R, resin composite; E, enamel; D, dentin.*

In this study, enamel etching was previously performed for both adhesive systems, and may have improved the micromechanical interlocking of resin tags in the conditioned enamel surface.[14,27] This procedure not only seals the restoration margins in the long term but also protects the dentin bond, which is more vulnerable to degradation.[28]

Images from OCT allowed differentiation between enamel, dentin, gaps, and resin composite (Figures 1 through 3). Clinically, OCT real-time visualization of tissue microstructure can prevent patients from the need to remove tissues and process specimens as well as from exposure to a radiation dose. OCT images differentiate the tissue optical properties, which include the effects of optical absorption and scattering.[29] Even carious demineralization can be seen in OCT through noninvasive and instant images.[20] Bonding to dentin has been a challenge when considering bond durability, as this substrate has significant structural and morphologic heterogeneity compared with enamel.[14,27,30]

The second hypothesis was partially accepted because there was a significant difference between the adhesive systems when bonded to dentin. The SB2 adhesive system showed greater gap percentages in dentin after TC compared with CSE, for all composites evaluated.

Table 2. *Mean Percentages and Standard Deviations of the Variation of Gaps at the Dentin/Restoration Interface Based on Thermal Cycling, Composite Resin, and Adhesive System*[a]

TC	Composite	Adhesive System	
		CSE	SB2
Before	AP	*4.20 ± 2.06 Aa	*4.90 ± 3.50 Aa
	VD	*6.05 ± 4.45 Aa	*7.70 ± 3.59 Aa
	Z250	*6.87 ± 2.97 Aa	*7.18 ± 3.05 Aa
After	AP	6.11 ± 2.65 Ba	11.34 ± 7.5 Aa
	VD	8.49 ± 5.90 Ba	17.51 ± 7.39 Aa
	Z250	10.26 ± 4.75 Ba	16.29 ± 6.73 Aa

[a] *Means followed by different letters (uppercase in horizontal and lowercase in vertical) differ from each other ($p \leq 0.05$) within the same group of cycling.*
** Differs from the mean after cycling in the same composite and adhesive system ($p \leq 0.05$).*

These results are in accordance with Frankenberger and others,[13] who observed that dentin bonding systems with a separate hydrophobic component, such as CSE, are less sensitive to thermomechanical cycling. Furthermore, the additional ionic bonding with residual hydroxyapatite is reached from etched dentin when a mild self-etching adhesive system is used.[27]

The better performance of CSE in dentin can be attributed to the presence of a 10-methacryloyloxydecyl dihydrogen phosphate (MDP) monomer in its composition. According to Nurrohman and others,[31] when CSE is used, hydroxyapatite crystals remain within the hybrid layer. The residual hydroxyapatite crystals can serve as a template for the chemical reaction with functional groups such as MDP.[32] In the chemical interaction of hydroxyapatite with the adhesive components, MDP bonds strongly to crystal, forming a stable link between calcium and MDP.[32,33] Therefore, MDP is a monomer that has hydroxyapatite affinity with chemical functional groups. After polymerization, monomers individually bond to the tooth's hydroxyapatite and form a polymer.[34]

When SB2 was used, a higher percentage of post-TC gaps can be attributed to dentin etching, which seems to dissolve and remove the natural protection of collagen, making the complex collagen-resin more vulnerable to degradation by water sorption, which is possibly increased by the process of thermal degradation during TC.[35] These two simultaneous stresses (water immersion and temperature changes) can destabilize the bond between adhesive system/tooth[27] and promote the hydrolytic degradation of resin components in the adhesive interface.[17]

Clinically, the etch-and-rinse technique leads to a question concerning the wettability of the dentin. It is not uncommon to have overly wet regions and overly dry surfaces in the same preparation, which causes a nonuniform resin bonding. Etch-and-rinse adhesive systems are more technique sensitive because optimal hybridization and sealing of dentinal tubules with the wet bonding technique may differ with each bonding system.[36] Although most bonded restorations are retained because there is sufficient well-bonded surface area, a common clinical manifestation of inconsistent bonding within a restoration is the patient's complaint of postoperative sensitivity.[37-39] Self-etching adhesives can surpass these problems because dentin must not be etched. It reduces postoperative sensitivity due to the decrease in hydraulic conductance through the dentinal tubules.[40,41]

The third hypothesis was rejected because none of the resin composite group comparisons showed a significant difference, either pre- or post-TC degradation.

This finding is in agreement with Baracco and others,[42] who compared *in vivo* restorations made with conventional and low-shrinkage resin composites. Those authors showed declining marginal adaptation scores in the restorations placed with all systems and concluded that the low-shrinkage resin composite used in their study provides adequate clinical performance but does not surpass the behavior of methacrylate-based conventional materials. On the other hand, Yamamoto and others[43] compared the polymerization stress of low-shrinkage and conventional resin composites, observing that the VD resin composite showed lower polymerization stress values compared with a conventional dimethacrylate resin composite. Finally, those authors concluded that, because of the many factors that influence polymerization stress development in resin composites, reduced shrinkage itself does not always generate lower stress[43] or higher levels of restoration marginal integrity.[42]

The results obtained in our study can be related to the depth of the cavity used. According to Braga and others,[44] there is a direct relationship between stress generated by polymerization shrinkage and the depth of a cavity. Those authors concluded that the volume shrinkage of the composite doubles as the depth of the cavity increases from 1 to 2 mm. Therefore, it is assumed that deeper cavities have higher shrinkage stress and, consequently, more marginal gaps.

In the present study, OCT was able to visualize gaps of composite restorations through instant and noninvasive images, corroborating the results of other recent studies.[10,11,18-21] In previous studies, a significant increase in the signal intensity (peak) at the tooth–restoration interfacial zone was confirmed, which appeared as bright clusters and indicated the loss of marginal seal.[17]

The deterioration of the interface in this present study is in accordance with the studies mentioned previously,[10,11,18-21] which had negatively affected the tooth/restoration marginal interface compared before and after TC.

The limited depth of viewing of the OCT device, which does not allow the visualization of deeper cavities, might be a limiting factor for this type of methodology. However, the cavity size used in this study is compatible and convenient with NCCL cavities.

From the results obtained in this study, future long-term studies on the marginal integrity of NCCL and composites with respect to TC and degradation should be conducted. Future improvements related to restoration depth analyses when using OCT will allow further research. Thus, OCT can be considered a nondestructive method for evaluating the stability of the enamel/restoration and dentin/restoration interfaces simultaneously, and it has potential for clinical use.

CONCLUSION

According to the results, the following can be concluded:

- TC affected marginal integrity only for dentin margins. The enamel/restoration interface remained stable even after TC, where enamel etching was performed before placement of either adhesive system;
- Dentin margins restored with the CSE adhesive system showed better marginal adaptation than those restored with SB2 when subjected to TC. Adhesive systems performed similarly on etched enamel;
- Resin composites did not influence the marginal integrity of NCCL restorations.
- OCT could distinguish tooth tissue (enamel and dentin) from resin composite, adhesive system and marginal gaps.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of Piracicaba Dental School – UNICA. The approval code issued for this study is #104/2012.

Conflict of Interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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