Microleakage and Micromorphology of the Resin-dentin Interface in Primary Molars Following Different Endodontic Irrigation Regimens

Burcu Togay* / Atila Ataç** / Zafer C. Cehreli***

The aim of this study was to evaluate the effect of two endodontic irrigants (2.5% sodium hypochlorite and 17% Ethylenediamintetraacetic acid) on the microleakage and micromorphology of the resin-dentin interface in pulpectomized primary molars. Standardized Class-II cavities were prepared in extracted, non-carious primary molars after which chemo-mechanical preparation of the root canals were performed in conjunction with K-Files and 2.5% sodium hypochlorite (NaOCl) between each file size. Final irrigation of the specimens were made with either 2.5 NaOCl, 17% Ethylenediamintetraacetic acid (EDTA) or with saline (control). The root canals were filled with a calcium hydroxide paste and the cavities were sub-grouped to be restored with one of the following materials: a:Amalgam, b: Prime&Bond NT (with prior total etch) and TPH resin-based composite, c: Prime&Bond NT and Dyract d: Prime&Bond NT (with Non-Rinse Conditioner pre-treatment); and e:Prompt L-Pop and F2000. Following thermocycling and 90-day water storage, the occlusal and gingival microleakage of restorations were assessed with dye penetration and image analysis. Separate specimens were processed for scanning electron microscopic investigation of the resin-dentin interface. The occlusal and gingival microleakage of the tested restorative systems was not affected by different irrigation regimens (p>0.05), while final restoration with Prime&Bond NT+TPH and Prompt L-Pop+F2000 resulted in the lowest microleakage values (p<0.05). Following the use of 17% EDTA for final flush, the latter two groups produced relatively thicker hybrid zones, when compared to the saline-irrigated specimens.

***Key Words**: Tooth, primary; Irrigation; Sodium Hypochlorite; EDTA; Microleakage; Hybrid zone; Image analysis*
J Clin Pediatr Dent 31(2):98-103, 2006

INTRODUCTION

Resin composites and compomers have become popular for the restorations of primary anterior and posterior teeth.[1] They are well-accepted due to their low relative thermal conductivity, preservation of the dental structure in cavity preparation, continuous progress in the stability of their composition, and because of the increasing demand from parents to provide esthetic restorations to their children.[2-5] Compomers also include the properties of the composites and glass ionomer cement and it is an esthetic material, light-cured and with efficient short-term inhibition of caries due to their fluoride release.[2]

For primary molars having undergone pulpotomy or pulpectomy, stainless-steel crowns (SSCs) are the most preferred restoration of choice.[6-9] Traditionally, SSCs have been recommended for restoration of such primary teeth, assuming full-crown coverage may provide less leakage compared to other restorative techniques.[8,9] However, this recommendation has not been supported by any controlled study to date. Moreover, even SSCs perfectly adapted on extracted teeth have been shown to demonstrate considerable cervical microleakage in vitro.[10]

The widely held clinical perception regarding the brittleness and susceptibility to fracture of pulpally treated teeth[11,12] is another major reason for the use of SSCs in primary molars.[13,14] Nevertheless, results of previous laboratory[12,14-17] and clinical work[18,19] have demonstrated that loss of a vital pulp, does not lead to progressive changes in the biomechanical properties of tooth structure that could render it more brittle. Contrary, the similarity between the biomechanical properties (i.e., punch shear strength, toughness, hardness, fracture resistance) of endodontically treated teeth and their vital counterparts suggest that other factors including loss of dentinal support (size of restoration), subsequent acute trauma or continuous flexure of tooth structure may be more critical to susceptibility to fracture.[12,14-20]

The placement of direct adhesive restorations has generally been found to have a strengthening effect on prepared teeth with few exceptions.[17-19] Adhesive restorations may have several advantages over SSCs in primary teeth including preservation of sound tooth tissue and normal contact area, and enhanced resistance to microleakage.[14] Indeed, the use of adhesive restorations over the root canal filling is gaining wider acceptance in endodontics.[18] Because endodontic sealer pastes and gutta-percha provide minimal resistance to bacterial leakage,[21,22] dentin bonding agents have been proposed as a secondary seal to prevent coronal microleakage, a significant cause of endodontic failure.[22,23] However, placement of an adhesive restoration after pulpectomy may present a potential problem, since irriga-

*Burcu Togay. Research Assistant, Department of Pediatric Dentistry, Faculty of Dentistry, Hacettepe University, Ankara, Turkey
**Atila Ataç. Associate Professor, Department of Pediatric Dentistry, Faculty of Dentistry, Hacettepe University, Ankara, Turkey
***Zafer C. Cehreli. Associate Professor, Department of Pediatric Dentistry, Faculty of Dentistry, Hacettepe University, Ankara, Turkey

Send all correspondence to: Zafer C. Cehreli, DDS, PhD, Gazi M. Kemal Bulvari 61/11, 06570 Maltepe, Ankara, Turkey

Fax: +90.312.3243190

E-mail: zcehreli@yahoo.com

tion solutions such as sodium hypochlorite (NaOCl) may interfere with the sealing of dentin bonding agents.[24,25]

The purpose of this study was, therefore, to investigate the occlusal and gingival microleakage, and the resin-dentin interface of adhesively-restored pulpectomized primary molars that received root canal irrigations with 2.5% NaOCl, 17% ethylenediaminte-traacetic acid (EDTA) and distilled water (control).

MATERIALS AND METHODS

Specimen Preparation

180 non-carious human primary molars were used in this study. The teeth were extracted for orthodontic reasons and were obtained under a protocol approved by the Institutional Review Board. Inclusion criteria stipulated that at least 2/3 of the roots were intact. Following surface debridement with a hand scaling instrument and cleaning with a rubber cup and slurry of pumice, the teeth were stored at 40ºC in 0.9% w/v NaCl, containing 0.002% sodium azide for a maximum of three months. One operator prepared standardized Class II cavities (bucco-lingual width= 3mm on the occlusal and gingival sides) with a #245 tungsten carbide bur in a high-speed handpiece under copious water spray. The gingival margin was finished 1 mm below the cemento-enamel junction, as verified subsequently under a stereomicroscope at 10x magnification. No bevels were added at any margin of the preparation. Upon removal of the roof of pulp chamber to gain access to the root canals, the pulp tissue was removed with a barbed broach (Dentsply-Maillefer, Balleigues, Switzerland) and working length was established 1.0 mm short of the apical foramen. The apical portion of roots were sealed with warm modeling wax and the root canals were instrumented manually to the same file size (#30 K-file,Dentsply-Maillefer) using the step-back technique. The teeth were randomly assigned into three groups (n=60/each) with regard to the irrigants used during the mechanical preparation of the root canals:

Group 1: (Control): Roots were irrigated with 5 ml saline solution after each instrument, and with 10ml of the same irrigant for final irrigation. A final flush was performed with 2 ml distilled water.

Group 2: Irrigation with 5 ml of 2.5% NaOCl after each instrument, and with 10ml of the same irrigant for final irrigation. A final flush with 2 ml distilled water was performed to remove NaOCl.

Group 3: Irrigation with 5 ml of 2.5% NaOCl at each instrument change, followed by 10 ml 17% EDTA (pH:7.4) for final irrigation. A final flush with 2 ml distilled water was performed to neutralize EDTA.

In all specimens, the irrigation was made with the cavity preparation facing downwards, so as to facilitate contact of the irrigant with cavity walls. Following mechanical preparation, the roots were filled with a calcium hydroxide-based paste with the spiral-lentulo technique. Excess paste was removed from the pulp chamber and the canal orifices were sealed with an approximately 2 mm-thick conventional glass ionomer cement base. Stainless steel Tofflemire matrices were custom-fitted to the prepared teeth and specimens in each irrigation group were further assigned randomly into five subgroups (n=12/each) to receive the following coronal restorations:

Group a: (negative control): A non-gamma II capsule amalgam (Permite II, SDI, Australia) was triturated in an amalgamator (Ultramat, SDI) according to the manufacturer's recommendations and condensed into the cavity in small increments. Following carving and burnishing, the teeth were wrapped in moist gauze and stored in separate vials in a 370C incubator for 24 hours(h) to allow setting of amalgam. Thereafter, the restorations were finished and polished with carbide finishing burs in combination with silicon polishing instruments.

Group b: Enamel and dentin surfaces were etched with 36% phosphoric acid gel (DeTrey Conditioner 36, De Trey, Germany; 30 seconds (s) enamel and 15 s dentin) and thoroughly washed there after with air-water jet for 15 s. Excess water was removed from the cavity by sterile cotton pellets, leaving the enamel and dentin surfaces visibly moist. An acetone-based single-bottle dentin adhesive (Prime&Bond NT, Dentsply, Germany) was applied in ample amounts on cavity walls with a microbrush and left undisturbed for 20s. The solvent was, then, removed with a gentle stream of compressed air. After light-curing for 10 s, a second layer of adhesive resin was applied, immediately air-thinned and light-cured in the same manner. A hybrid resin composite material (TPH, Dentsply, Konstanz, Germany) was placed into the cavities, with a maximum increment of thickness of 2mm. Each increment received 40s photopolymerization. The restorations were additionally light-cured from buccal and lingual sides following removal of the matrix band. Anatomic contouring and finishing of the final restoration were accomplished with carbide and ultrafine diamond finishing burs, cups and aluminum oxide disk series (Soflex, 3M/ESPE, Seefeld, Germany), immediately after light curing.

Group c: Prime & Bond NT was applied into the cavity as with Group b without prior acid etching. A polyacid-modified resin composite (compomer) material (Dyract AP, Dentsply, Konstanz, Germany) was placed, photopolymerized and finished in accordance with the restorative protocol followed in Group b.

Group d: A non-rinse conditioner (NRC, Dentsply, Germany) was applied on cavity walls with a microbrush, left undisturbed for 20 seconds, and air-dried for 5 seconds. Thereafter, Prime & Bond NT was applied into the cavity as with Group b. Finally, the compomer Dyract AP was placed, photopolymerized and finished in accordance with the restorative protocol followed in Group b.

Group e: A predosed, all-in-one self-etch adhesive (Prompt L-pop, 3M/ESPE, Seefeld, Germany) was activated by squeezing the two components and the resulting mix was applied directly to the cavity walls with a gentle rubbing action. After 15 seconds, the solvent was evaporated with a mild air flow and light cured for 10 seconds. A polyacid-modified resin composite material (F2000, 3M/ESPE, Seefeld, Germany) was placed, photopolymerized and finished as with Group b.

Specimens were stored in water at 37ºC for 48 hours after which thermal cycling was performed in distilled water at 5-55±20C for 1000 cycles (dwell time: 30s). The teeth were, then, stored in distilled water at room temperature for 90 days.[26] The water was changed every week.[27]

Microleakage test and image analysis

The root apices were sealed with sticky wax to prevent dye penetration. The samples were coated with two consecutive layers of nail varnish up to 1mm from the restoration margins. Samples were, then, immersed in 0.5% basic fuchsin solution (Wako Pure Chemical Industry, Osaka, Japan) for 24 hours. Thereafter, samples were rinsed thoroughly under tap water, air-dried and embedded in a phenolic ring with epoxy resin (Struers, Copenhagen, Denmark). Three parallel longitudinal sections were made through the resin restorations using a low-speed, water-cooled diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) in the mesio-distal direction. For each specimen, dye penetration along the occlusal and gingival margins on each of the three sectioned surfaces was digitally photographed at 20x (1280x1024 resolution) under a stereo-microscope (Olympus, Tokyo, Japan) and transferred to an IBM-compatible PC. On each section, the staining along both occlusal and gingival restoration interfaces was measured (in mm) using image analysis software (ImageJ V.134, National Institutes of Health, Bethesda, Maryland). The occlusal and gingival microleakage (mm) of each specimen was recorded by calculating the mean occlusal and gingival microleakage values of three sections. For each sub-group, the differences between the extent of dye penetration at the occlusal and gingival margins were evaluated statistically using Wilcoxon Signed Ranks test with significance set at P=0.05. The occlusal and gingival microleakage values of the sub-groups were compared statistically with Kruskal-Wallis Test at the same level of significance.

Scanning Electron Microscopy (SEM) Evaluation

For each sub-group, three additional teeth were prepared and sectioned in the same manner described before in order to evaluate the tooth-adhesive inteface under SEM. The sections were first exposed to 6 N HCl for 15 s followed by 1% NaOCl for 10 min, and dehydrated in ascending grades of ethanol (30, 50, 95% for 30 min each, and 100% for 60 min). After the final ethanol bath, the specimens were dried by immersion in hexamethyldisilazane (HMDS, Electron Microscopy Sciences, Hatfield, PA) for 30 min, placed on a filter paper inside a covered glass vial, kept in vacuum for 24 h. Subsequently, the specimens were sputter coated with gold-palladium (Balzers-SCd 050 sputter coater, Germany) for observation with the use of an SEM (JSM-6400 V, JEOL, Tokyo, Japan) at 20 kV of accelerating voltage.

RESULTS

Microleakage Evaluation

Representative specimens from different groups depicting microleakage are presented in Figure 1. The microleakage values (in mm) of the test groups are presented in Table 1 as mean±SD. Regardless of the irrigation solution used, all groups demonstrated microleakage to varying degrees. When saline was used as an irrigant, group 1b (Total Etch+ Prime&Bond NT+TPH) showed significantly less occlusal microleakage ($p<0.05$) than groups 1a, 1c and 1d (Amalgam, Prime& Bond NT+Dyract and NRC+Prime&Bond NT+Dyract, respectively). Although restoration with Total Etch+ Prime&Bond NT+TPH (group 1b) yielded the lowest occlusal microleakage value, this was not significantly different than that of the Prompt L-Pop+F2000 group (group 1e, $p>0.05$). For gingival microleakage, the Total Etch+ Prime&Bond NT+TPH and Prompt L-Pop+F2000 groups (groups 1b and 1e, respectively) demonstrated significantly less values than that of amalgam and NRC+Prime&Bond NT+Dyract ($p<0.05$).

When 2.5% NaOCl was used as an irrigant, the Total Etch+ Prime&Bond NT+ TPH group (group 2b) demonstrated significantly less occlusal microleakage values ($p<0.05$) than those of all other restorative groups (groups 2a,2c,2d, and 2e). However, for gingival microleakage values, only those of the Total Etch+ Prime&Bond NT+TPH and Prompt L-Pop+F2000 groups (groups 2b and 2e) were significantly lower than the amalgam group (group 2a, $p<0.05$). Similar to 2.5% NaOCl-irrigated samples, the Total Etch+ Prime&Bond NT+ TPH group (group 3b) demonstrated significantly less occlusal microleakage values ($p<0.05$) than those of all other restorative groups (groups 3a,3c,3d, and 3e), when 17% EDTA was used for final flush. In regards to gingival microleakage values following EDTA irrigation, group 3e (Prompt L-Pop+F2000) demonstrated significantly less values than that of amalgam and

TABLE 1: Occlusal and gingival microleakage of the test groups. Values (mm) are presented as mean±SD.

Irrigant/ Group (n=60)	Sub-group (n=12)	Restoration	Occlusal	Gingival
Saline (Control)/ Group 1	a	Amalgam	1.19±1.09	1.94±0.73
	b	Total Etch+ Prime&Bond NT + TPH	0.19±0.08	1.00±0.78
	c	Prime& Bond NT + Dyract	0.91±0.71	1.76±1.39
	d	NRC+Prime&Bond NT + Dyract	0.70±0.33	1.66±0.73
	e	Prompt L-Pop + F2000	0.47±0.19	1.37±1.01
2.5% NaOCL/ Group 2	a	Amalgam	1.21±1.00	1.96±0.75
	b	Total Etch+ Prime&Bond NT + TPH	0.18±0.06	1.23±0.78
	c	Prime& Bond NT + Dyract	0.90±0.74	1.78±1.37
	d	NRC+Prime&Bond NT + Dyract	0.71±0.34	1.72±0.74
	e	Prompt L-Pop + F2000	0.48±0.30	1.47±1.07
17% EDTA/ Group 3	a	Amalgam	1.20±1.10	1.92±0.72
	b	Total Etch+ Prime&Bond NT + TPH	0.16±0.05	1.03±0.78
	c	Prime& Bond NT + Dyract	0.89±0.75	1.73±1.35
	d	NRC+Prime&Bond NT + Dyract	0.68±0.13	1.64±0.78
	e	Prompt L-Pop + F2000	0.45±0.29	1.35±1.08

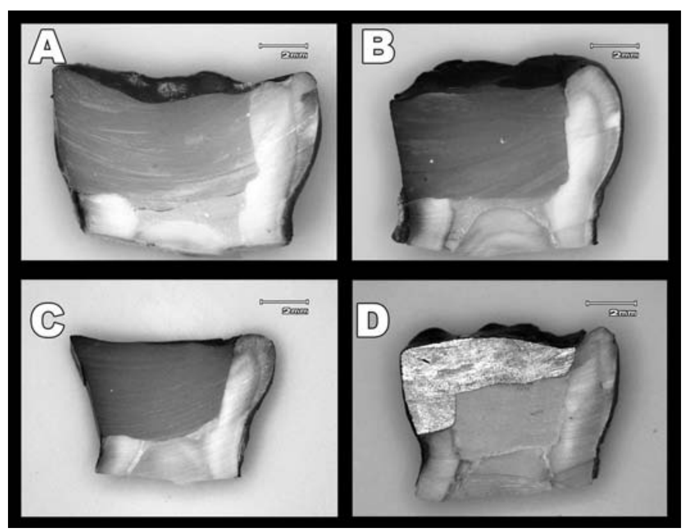

FIGURE 1: Representative micrographs of specimens showing microleakage at the tooth-restoration interface (Original magnification, 20X, Scale Bar = 2mm). A. Group 3b (Upper left), B. Group 2c (Upper Right), C. Group 3d (Lower left), and D. Group 2e (Lower right).

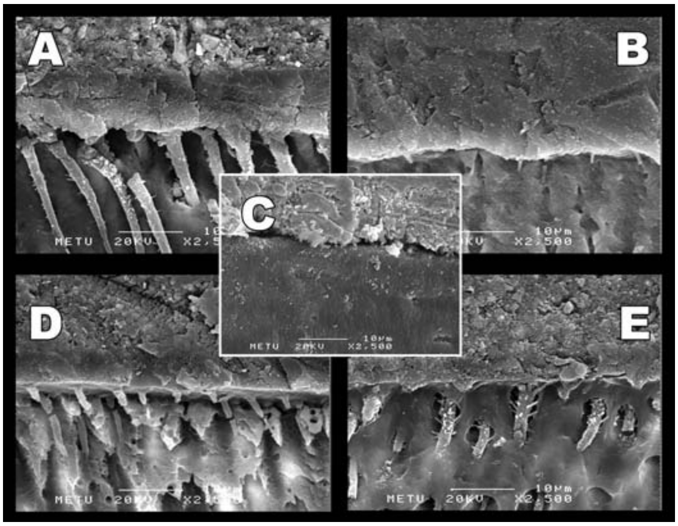

FIGURE 2: Representative SEM micrographs of the tooth-restoration interface in control (saline-irrigated) samples. A. Prime&Bond NT (with prior total etch)+TPH, B.Prime&Bond NT+Dyract, C.Amalgam, D.Prime&Bond NT (with prior NRC pre-treatment), and E.Prompt L-Pop+F2000.

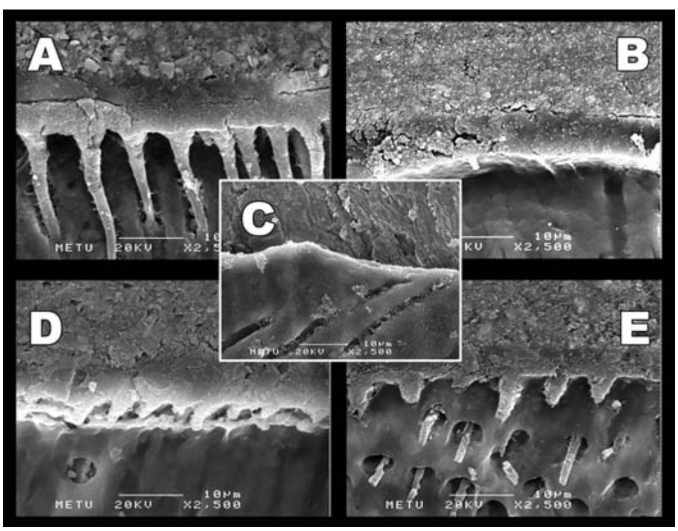

FIGURE 3: Representative SEM micrographs of the tooth-restoration interface in 2.5% NaOCl-irrigated samples. A. Prime&Bond NT (with prior total etch)+TPH, B.Prime&Bond NT+Dyract, C.Amalgam, D.Prime&Bond NT (with prior NRC pre-treatment), and E.Prompt L-Pop+F2000.

NRC+Prime&Bond NT+Dyract (groups 3a and 3d, respectively; $p<0.05$).

When the experimental sub-groups were compared statistically for the effect of irrigation on microleakage, no significant difference was found for either the occlusal or gingival values ($p>0.05$). At both margins, the microleakage of the tested restorative systems was not affected by different irrigation regimens. Regardless of the irrigation regimens tested, comparison of occlusal and gingival microleakage within each sub-group showed that, prior etching of cavity margins with phosphoric acid (groups 1b, 2b, 3b) and NRC (groups 1d, 2d, 3d) yielded significantly lower dye penetration at the occlusal (enamel) margins compared to the gingival margins (Wilcoxon Signed Ranks test, $p<0.05$). For all sub-groups employing Prompt L-Pop, Prime&Bond NT (without any prior conditioning) and amalgam, there was no significant difference between the

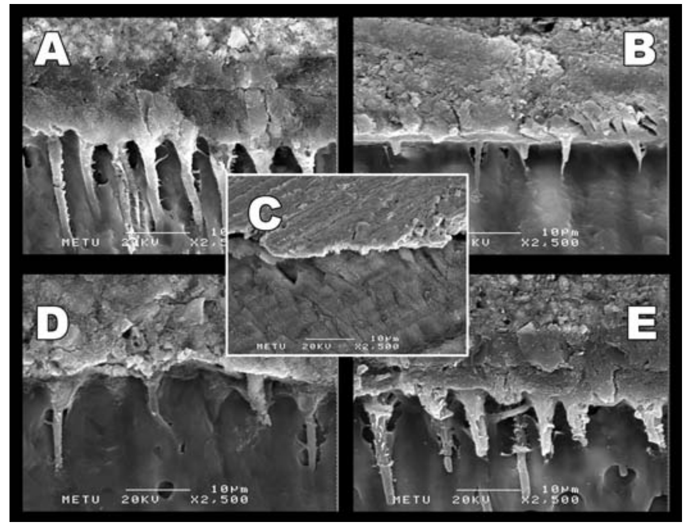

FIGURE 4: Representative SEM micrographs of the tooth-restoration interface in 17% EDTA-irrigated samples. A. Prime&Bond NT (with prior total etch)+TPH, B.Prime&Bond NT+Dyract, C.Amalgam, D.Prime&Bond NT (with prior NRC pre-treatment), and E.Prompt L-Pop+F2000.

microleakage values at the occlusal and gingival margins ($p>0.05$).

SEM evaluation

Representative micrographs of the resin-dentin and amalgam-dentin interface of specimens which received prior saline irrigation (control) are presented in Figure 2. Application of total etch and Prime&Bond NT resulted in an approximately 5-7µm-thick, continuous hybrid layer with well-defined resin tags demonstrating thin resin projections, indicative of lateral tubule hybridization (Figure 2a). There was no sign of hybridization when Prime&Bond NT was used alone and only a few, thin resin tags were present (Figure 2b). The amalgam-dentin interface showed signs of incomplete adaptation, as evidenced by several gaps (Figure 2c). Application of NRC and Prime&Bond NT produced a thin hybrid zone with relatively more tubular projections than that observed when the same adhesive was used alone (Figure 2d). Prompt L-Pop produced a uniform and continuous hybrid zone (approximately 3-5µm-thick) with thick resin tags, characterized by lateral tubule hybridization (Figure 2e).

In general, irrigation of the dentin with 2.5% NaOCL did not alter the morphological features of the resin-dentin and amalgam-dentin interfaces (Figure 3). In the amalgam group, more regions of complete adaptation were observed (Figure 3c). In addition, a few sites of submicron hiati were detected in the NRC+Prime&Bond NT group (Figure 3d). Following the use of 17% EDTA for final flush, the total etch and Prime&Bond NT (b) and Prompt L-Pop (e) groups produced relatively thicker hybrid zones, when compared to the saline-irrigated specimens (Figures 4a and 4e). Application of Prime&Bond NT alone and with prior NRC pretreatment produced a continuous hybrid zone-like interface, with only minor improvements in the number and thickness of resin tags (Figures 4b and 4d). Similar to NaOCL-treated samples, the adaptation of amalgam to primary dentin was good, but there were still signs of microgaps (Figure 4c).

DISCUSSION

Microleakage test is a widely used method to evaluate the sealing performance of adhesive systems in dentin and enamel.[28-30]

Among different methods employed, measurement of dye penetration on sections of restored teeth is the most commonly used technique.[29,30] In the present study, three sections were made through each restoration to increase the reliability of measurements.[29] This technique was combined with image analysis in order to obtain quantitative results instead of a conventional subjective scoring. A relative merit of this objective approach compared with a subjective scoring system was to discard the need for scoring by separate evaluators and for consensus scoring in borderline cases, as well as statistical procedures with regard to inter-examiner reliability.

One of the primary requirements for any dentin bonding system is resistance to degradation in the oral environment.[31] If water absorption occurs at the adhesive/dentin interface, there may be fluid movement at the junction of the adhesive resin and hybrid layer during flexing of the restoration and tooth.[32] Coupled with the degradation of the adhesive phase that leaves previously hybridized collagen unprotected,[33] the cumulative effect would be breakdown of the adhesive/dentin bond, and ultimately, undermining of the marginal integrity of the adhesive restoration.[26] In addition to thermocycling, a widely used aging technique that significantly impairs marginal sealing,[34] the specimens herein were subsequently water-aged for 90 days[26] in an attempt to enable quantitative evaluation of microleakage after prolonged degradation of the resin-dentin bonds. Recent work has provided direct evidence of the breakdown of hydrolytically unstable adhesive components at the hybrid layer/dentin interface in class II composite restorations, following 90 days of aqueous aging.[26]

NaOCl is a well-known non-specific proteolytic agent capable of removing organic material, as well as magnesium and carbonate ions.[35,36] Applied in various concentrations (0.5-5.25%), NaOCl is the most favored endodontic irrigant because of its tissue-dissolving, antimicrobial and lubricating properties.[37] Studies conducted on extracted permanent teeth have reported that NaOCl irrigation may adversely affect the sealing properties of total- and self-etch adhesives at gingival (resin-dentin) margins.[24,25] In contrast to those reports, however, sealing ability of the tested systems at the gingival margins were not affected by NaOCl irrigation in the present study. This finding can be explained by the lower concentration of NaOCl employed (half the concentration used in permanent teeth) herein.[25,40] Moreover, the duration of chemo-mechanical preparation, and thus, the time of contact of NaOCl with cavity margins was shorter (approximately 20 min) than that reported in permanent teeth previously.[25] Higher concentration and longer treatment time may result in more significant changes dentin,[25] as well as increased possibility of mineral loss,[35,36] that may render the dentin substrate less ideal for adhesive procedures.[38]

Several authors have recommended the sequential use of organic and inorganic solvents as endodontic irrigants, since no single solution has yet proved to be capable of removing the smear layer alone.[39] In this regard neutral EDTA solutions in a 15-17% concentration, has gained wide acceptance as a final flush solution (in association with NaOCl) in the removal of intracanal smear layer and smear plugs.[39,40] In the present study, microleakage at the occlusal and gingival margins of the test groups that received EDTA irrigation was not significantly different than those of the saline (control) and NaOCl-irrigated groups. Due to the lack of published data in primary and permanent teeth, comparisons cannot be made. However, it can be speculated that the short duration of irrigation (approximately 1 minute), coupled with the neutralizing effect of final irrigation with distilled water may have significantly limited the etching effect of EDTA to the removal of smear layer only.[41] Recent work has shown that application of 15% EDTA for 1 minute is as effective as 3 and 5 minute applications of the same irrigant in the removal of intracanal smear layer.[41]

Studies conducted on extracted permanent teeth have reported that some irrigation solutions including NaOCl cause significantly more microleakage at the gingival (resin-dentin) margins of resin-bonded restorations compared to the enamel margins.[24,25] Reportedly, this is not an unexpected outcome since in both dentitions, the marginal integrity of the enamel-resin bond has been shown to be superior to its dentin/cementum counterpart.[1,30,42,43] However, the present study showed that this difference would depend on the type of adhesive/restorative system employed, rather than the chemicals used for irrigation. Only the phosphoric acid and NRC pre-treated groups demonstrated significantly less microleakage at enamel margins than that along the gingival margins, and the observation of this finding in all tested irrigation regimens cannot be considered coincidental. Contrary, the difference between occlusal and gingival microleakage values for Prompt L-Pop were not significantly different, but at both margins, the adhesive system yielded a seal that was equivalent to or significantly better than phosphoric acid and NRC pre-treated groups, respectively (Table 1).

As observed under the SEM, the morphological appearances of the resin-dentin interface of the adhesive systems tested herein are in line with previous reports. Typically, Prime&Bond NT and Prompt L-Pop produced a continuous hybrid layer with well-defined resin tags, and even lateral tubule hybridization. The relatively-thicker hybrid zone observed in the latter two groups for EDTA-irrigated samples can be explained by the possible total removal or reduction of smear layer by EDTA prior to application of both adhesive systems.[40] Accordingly, the etching gel or the low-pH self etching primer may penetrate deeper in the absence of (or presence of a very thin) smear layer,[44] within the same period of time which the acid in other groups (control and NaOCl) had to overcome the obstacle of smear before it could reach and interact as deep with the underlying dentin.[45] There is, however a general consensus that the quality of dentin hybridization is more important than the actual thickness of the hybrid layers in establishing the long-term seal of bonded restorations.[46] Consequently, the depth of of dentin demineralization may not be an important factor for dentin adhesion.[38,46]

Regardless of the irrigation regimen employed, none of the adhesive systems tested within the experimental conditions of this study were able to totally prevent the occurrence of microleakage along the enamel and gingival margin of primary molars. At both margins, Prime&Bond NT (alone) and amalgam displayed the worst sealing performance. In vitro simulations of clinical conditions under which enamel and dentin bonding would fail, such as thermocycling and water storage are difficult, because the factors involved in bond degradation *in vivo* are numerous, and not completely known.[30] In an attempt to delay the effects of inevitable post-operative microleakage, the results obtained herein can only be extrapolated cautiously to the clinical situation for Prime&Bond NT with prior total etch and Prompt-L-Pop with all margins surrounded by enamel (Class I cavities) and preferably supersealed with a bonding resin. The question is whether these two adhesive systems, as well as other available adhesive restorations, in conjunction with their resin-based restoratives can provide a long term seal along the gingival margin *in vivo*.

Thus, clinical studies, even though they are time-consuming, expensive, and lack control over important variables, are necessary.

REFERENCES

1. Casagrande L, Brayner R, Barata JS, de Araujo FB. Cervical microleak age in composite restorations in primary teeth-in vitro study. J Dent; 33:627-638. 2005
2. Garcia-Godoy F. Resin-based composites and compomers in primary molars. Dent Clin North Am;44:541-570. 2000
3. Tran LA,Messer LB. Clinicians' choices of restorative materials for children. AustDent J;48:221–32. 2003
4. Ram D, Fuks AB. Clinical performance of resin-bonded composite strip crowns in primary incisors: a retrospective study. Int J Paediatr Dent;16:49-54. 2006
5. Roeters J, Frankenmolen F, Burgersdijk R, Peters T. Clinical evaluation of dyract in primary molars: three-year results. Am J Dent;11:143–8. 1998
6. Randall RC, Vrijhoef MMA, Wilson NHF. Efficacy of preformed metal crowns vs. amalgam restorations in primary molars: a systematic review. J Am Dent Assoc; 131: 337-343. 2000
7. Dawson LR, Simon JF Jr, Taylor PP. Use of amalgam and stainless steel restorations for primary molars. J Dent Child;46:420-422. 1981
8. Croll TP. Preformed posterior stainless steel crowns : An update. Compendium; 20:89-104. 1999
9. Guelmann M, McIlwain MF, Primosch RE. Radiographic assessment of primary molar pulpotomies with resin-based materials. Pediatr Dent;27:24-27. 2005
10. Guelmann M, Bookmyer KL, Villalta P, Garcia-Godoy F. Microleakage of restorative techniques for pulpotomized molars. J Dent Child;71:209-211. 2004
11. Helfer AR, Melnick S, Schilder H. Determination of the moisture content of vital and pulpless teeth. Oral Surg Oral Med Oral Pathol; 34:661-670. 1972
12. Sedgley CM, Messer HH. Are endodontically treated teeth more brittle? J Endod; 18:332-335. 1992
13. Davina AB. A conservative approach to the pulpotomy in primary teeth. J Clin Pediatr Dent; 22:103-105. 1998
14. El-Kalla IH, Garcia-Godoy F. Fracture strength of adhesively restored pulpotomized primary molars. J Dent Child; 66:238-242. 1999
15. Trope M, Tronstad L. Resistance to fracture of endodontically treated premolars restored with glass ionomer cement or acid etch composite resin. J Endod; 17:257-259. 1991
16. Papa J, Cain C, Messer HH. Moisture content of vital vs endodontically treated teeth. Endod Dent Traumatol; 10:91-93. 1994
17. Hernandez R, Bader S, Boston D, Trope M. Resistance to fracture of endodontically treated premolars with new generation dentine bonding systems. Int Endod J; 27:281-284. 1994
18. Manocci F, Bertelli E, Sheriff M, Watson TF, Ford TRP. Three-year clinical comparison of survival of endodontically treated teeth with either full cast coverage or with direct composite restoration. J Prosthet Dent; 88:297-301. 2002
19. Nagasiri R, Chitmongkolsuk S. Long-term survival of endodotically treated molars without crown coverage: A retrospective cohort study. J Prosthet Dent; 93:164-170. 2005
20. Farik B, Munksgaard EC. Fracture strength of intact and fragment bonded teeth at various velocities of the applied force. Eur J Oral Sci; 107:70-73. 1999
21. Magure ME, Kafrawy AH, Brown CE, Newton CW. Human saliva coronal microleakage in obturated root canals: An in vitro study. J Endod;17:324-31. 1991
22. Trope M, Chow E, Nissan R. In vitro endototxin penetration of coronally unsealed endodontically treated teeth. Endod Dent Traumatol;11:90-4. 1995
23. Belli S, Zhang Y, Pereira PNR, Pashley DH. Adhesive sealing of the pulp chamber. J Endod;27:521-526. 2001
24. Morris MD, Lee KW, Agee KA, Bouillaguet S, Pashley DH. Effects of sodium hypochlorite and RC-Prep on bond strengths of resin cement to endodontic surfaces. J Endod;27:753-757. 2001
25. Turkun M, Sevgican F, Aran B. Effect of endodontic irrigants on microleakage of coronal restorations. Am J Dent;18:353-358. 2005
26. Spencer P, Wang Y, Bohaty B. Interfacial chemistry of moisture-aged class-II composite restorations. J Biomed Mater Res Part B: Appl Biomater;77B: 234–240. 2006
27. Kitasako Y, Burrow MF, Nikaido T, et al. The influence of storage solution on dentin bond durability of resin cement. J Dent;16:1-6. 2000
28. Abo T, Uno S, Sano H.Comparison of bonding efficacy of an all-in-one adhesive with a self-etching primer system. Eur J Oral Sci;112;286-292. 2004
29. Raskin A, Tassery H, D'hoore W, Gonthier S, Vreven J, Degrange M, Dejaou J. Influence of the number of specimens on reliability of in vitro microleakage evaluations. Am J Dent;6:207-210. 2003
30. Osorio R, Toledano M, Osorio E, Aguilera FS, Tay FR. Effect of load cycling and in vitro degradation on resin-dentin bonds using a self-etching primer. J Biomed Mater Res A.;72:399-408. 2005
31. Kugel G, Ferrari M. The science of bonding: From first to sixth generation. J Am Dent Assoc;131:20S–25S. 2000
32. Sano H, Yoshikawa T, Pereira PNR, Kanemura N, Morigami M, Tagami J, Pashley DH. Long-term durability of dentin bonds made with a self-etching primer, in vivo. J Dent Res;78:906–911. 1999
33. Hashimoto M, Ohno H, Kaga M, Endo K, Sano H, Oguchi H. In vivo degradation of resin– dentin bonds in humans over 1 to 3 years. J Dent Res;79:1385–1391. 2000
34. Gale MS, Darwell BW. Thermal cycling procedures for laboratory testing of dental restorations. J Dent;27:89-99. 1999
35. Inaba D, Ruben J, Takagi O, Arends J. Effect of sodium hypochlorite treatment on remineralization of human dentine in vitro. Caries Res;30:218-224. 1996
36. Sakae T, Mishima H, Kozawa Y. Changes in bovine dentin mineral with sodium hypochlorite treatment. J Dent Res;67:1229-1234. 1988
37. Zehnder M. Root canal irrigants. J Endod 32:389-398. 2006
38. Perdigao J, Lopes M, Geraldeli S, Lopes GC, Garcia-Godoy F. Effect of a sodium hypochlorite gel on dentin bonding. Dent Mater;16:311-323. 2000
39. Stewart GG. A Scanning electron microscopic study of the cleansing effectiveness of three irrigating modalities on the tubular structure of dentin. J Endod; 24: 485-86. 1998
40. Altundasar E, Ozcelik B, Cehreli ZC, Matsumoto K. Ultramorphological and Histochemical Changes After ER,CR:YSGG Laser Irradiation and Two Different Irrigation Regimes. J Endod.;32:465-468. 2006
41. Teixeira CS, Felippe MCS, Felippe WT. The effect of application time of EDTA and NaOCL on intracanal smear layer removal: an SEM analysis. Int Endod J; 38:285-90. 2005
42. Barata JS, Araujo FB, Costa CAS, Garcia-Godoy F. Clinical radi ographical and histological evaluation of total etch technique in primary teeth. J Dent Res;76(SI):305. 1997
43. Fuks AB, Pulp therapy for the primary and young permanent dentitions. Dent Clin North Am;44:571-596. 2000
44. Perdigao J, Eiriksson S, Rosa BT, Lopes M, Gomez G. Effect of calcium removal on dentin bond strengths. Quintessence Int;32:142-146. 2001
45. Cehreli ZC, Yazici AR, Akca T, Ozgunaltay G. A morphological and micro-tensile bond strength evaluation of a single-bottle adhesive to caries-affected human dentine after four different caries removal techniques. J Dent.;31:429-35. 2003
46. De Munck J, Van Landuyt K, Peumans M, Poitevin A, Lambrechts P, Braem M, Van Meerbeek B. A critical review of the durability of adhesion to tooth tissue: methods and results. J Dent Res;84:118–132. 2005