©Operative Dentistry, 2007, **32-2,** 174-179

Restoration Interface Microleakage Using Two Total-etch and Two Self-etch Adhesives

S Deliperi • DN Bardwell • C Wegley

Clinical Relevance

The common believe that simpler, speedier, more user-friendly adhesives always reduce technique sensitivity should be revaluated. Clinicians should resist the attraction of new, faster, easier-use materials and ask for laboratory and clinical studies before switching to a new material.

SUMMARY

This study evaluated the efficacy of a total-etch and three self-etch adhesives in reducing microleakage after three months water storage and thermocycling.

Thirty freshly extracted caries-free human premolars and molars were used. Class V standardized preparations were performed on the facial and lingual surfaces, with the gingival margin placed 1 mm below the CEJ. The teeth were randomly divided into four groups; Group I: Xeno III one-step self-etch adhesive (Dentsply/Caulk), Group II: Prime & Bond NT total-etch adhesive (Dentsply/Caulk), Group III: i-Bond one-step self-etch adhesive (Heraeus Kulzer) and Group IV: Clearfil SE Bond two-step self-etch adhesive (Kuraray Medical). The teeth were restored using 2 mm increments of shade A2 resin composite (Esthet-X, Dentsply/Caulk). Each layer was cured using the Spectrum 800 curing light (Dentsply/Caulk) for 20 seconds at 600mW/cm^2. The teeth were stored in distilled water for 90 days. Samples were thermocycled 500x between 5°C and 55°C with a dwell of 30 seconds, then placed in a 0.5% methylene blue dye solution for 24 hours at 37°C. Samples were sectioned longitudinally and evaluated for microleakage at the occlusal and gingival margins under a stereomicroscope at 20x magnification. Dye penetration was scored: 0=no penetration; 1=partial dye penetration along the occlusal or gingival wall; 2=dye penetration along the occlusal or gingival wall; 3=dye penetration to and along the axial wall.

A Mann-Whitney test was used to demonstrate significantly more dye penetration in Group III than in the other groups at both the occlusal and gingival scores (*p*<0.0001). When comparing the

*Simone Deliperi, DDS, visiting instructor and research associate, Postgraduate Esthetic Dentistry, Tufts University School of Dental Medicine, Boston, MA, USA and private practice, Cagliari, Italy

David N Bardwell, DMD, MS, associate clinical professor of Restorative Dentistry, Tufts University School of Dental Medicine, Boston, MA, USA

Christopher Wegley, former undergraduate student, Tufts University School of Dental Medicine, Boston, MA, USA

*Reprint request: via G Baccelli, 10b/09126, Cagliari, Italy; e-mail: simone.deliperi@tufts.edu

DOI: 10.2341/06-54

occlusal and gingival scores for each group, the Wilcoxon Rank test showed no significant difference in dye penetration for Xeno III (*p*>0.05), Prime & Bond NT (*p*=0.059) and I Bond (*p*=0.083), and Clearfil SE Bond yielded more dye penetration at the occlusal than at the gingival wall (*p*=0.001).

INTRODUCTION

In the early 1990s, the introduction of the three-step total-etch adhesive system represented a revolution in adhesive dentistry. Once dentin is etched with phosphoric acid and the etchant is rinsed off, hydrophilic primers are used before applying a uniform layer of hydrophobic resin to complete hybridization.[1] However, the recent trend in adhesive products is to simplify the process into two steps or even one (single) step to make user-friendly and time saving products. Two-step total-etch adhesive systems and two-step self-etch adhesives were introduced into the market in the late 1990s.

In two-step total-etch systems, a separate etch and rinse phase is still involved, but a hydrophilic primer and hydrophobic resin are combined into one application. Although increased technique sensitivity is reported[1-2] for total-etch adhesives, a similar clinical performance is achieved for both conventional and simplified total-etch adhesive versions.[3-4]

Self-etch adhesives represent an alternative approach in enamel-dentin bonding. They do not require a separate acid etch step and are based on the use of non-rinse acidic monomers that simultaneously condition and prime dentin and enamel.[1,5-6] This approach eliminates the rinsing phase and does not require application of the primer in particular conditions of wetness due to the self-etch adhesives' water content; reduced technique-sensitivity and the risk of making errors during application are achieved. For these reasons, their popularity is increasing.[7]

Two-step self-etch adhesives are based on the separate application of self-etch primer and hydrophobic resin; they usually use a mild self-etch primer. Mild self-etch systems (pH≤2) are able to partially remove the smear layer and penetrate the dentinal surface, creating a less pronounced resin tag formation and hybrid layers that are thinner than those of total-etch systems. Very high dentin bond strengths, comparable to those obtained with total-etch adhesives, are reported;[8-9] conversely, a common concern is their inability to etch enamel to the same depth as phosphoric acid.[10]

Lately, further simplification has been achieved by introducing single-step self-etch adhesives that combine self-etch primer and hydrophobic resin into one application. For this reason, these single-step self-etch adhesives are also called "all-in-one" adhesive systems. They may have either mild or strong acidity. Strong self-etch adhesives (pH≤1) have been documented with ultra-morphological characteristics very similar to those produced by total-etch adhesives; their mechanism of bonding to dentin resembles that reported for total-etch adhesives with typical hybridization achieved by total-etch adhesives and the abundant formation of resin tags. However, all the materials in this group are characterized by increased hydrophilicity and water permeability; they are reported to act as semi-permeable membranes that permit the diffusion of water molecules from dentin across the adhesive interface.[11-13]

The objective of this study was to test the null hypothesis that two "all-in-one" adhesives do not perform better than a two-step self-etch and two-step total-etch adhesives in reducing microleakage; another objective was to determine whether the tested adhesives would perform as well on enamel as dentin.

METHODS AND MATERIALS

Thirty freshly extracted, caries free human premolars and molars were kept in distilled water at 4°C for 24 hours. The preparations were standardized as 4 mm long, 3 mm wide and 2 mm in depth and placed either on the facial or lingual surface of each tooth. Class V cavities were prepared, with the gingival margin 1 mm below the CEJ, using a #4 round bur (Brasseler, Savannah, GA, USA) with a high speed handpiece and copious amounts of water. No bevels were placed. The teeth were randomly divided into four groups corresponding to each adhesive system (Table 1). Fifteen teeth were assigned to each group.

In Group I, a one-step self-etch adhesive system (Xeno III, Dentsply/Caulk Mildford, DE, USA) was applied on dried dentin and enamel surfaces according to the manufacturer's instructions.

In Group II, each prepared tooth was etched with 34% H_3PO_4 (Tooth Conditioner Gel-Dentsply/Caulk) for 15 seconds, rinsed for 20 seconds, then gently blown to remove excess water, being careful to maintain a moist surface; a total etch nanofilled acetone-based adhesive system (Prime & Bond NT- Dentsply/Caulk) was applied according to the manufacturer's instructions.

In Group III, a one-step self-etch adhesive system (i-Bond, Heraeus Kulzer, Armonk, NY, USA) was applied on dried enamel and dentin according to the manufacturer's instructions.

Table 1

Group	Adhesive System	Manufacturer
I	Xeno III	Dentsply/Caulk
II	Prime & Bond NT	Dentsply/Caulk
III	i-Bond	Heraeus Kulzer
IV	Clearfil SE Bond	Kuraray Medical

In Group IV, a two-step self-etch adhesive system (Clearfil SE Bond, Kuraray Medical, Osaka, Japan) was applied on dried enamel and dentin according to the manufacturer's instructions.

The restorations were completely filled using wedge-shaped shade A2 composite increments (Esthet-X, Dentsply/Caulk), with each layer not being more than 2 mm thick, and cured for 20 seconds at 600mW/cm^2 using a Quartz-Tungsten-Halogen light (Spectrum 800, Dentsply/Caulk). The curing light built-in radiometer was used to check for light efficiency before starting each restoration. The restorations were finished using carbide burs and polished using a one-step diamond micro-polisher system (Pogo, Dentsply/Caulk). All the teeth were stored in distilled water at 37°C for three months.

The restored teeth were thermocycled 500x at a temperature of 5°C and 55°C. The dwell time was 30 seconds in each water bath, with a transfer time of 30 seconds between each bath. The samples were then blotted dry with a paper towel and the roots were sealed with sticky wax. An acid-resistant varnish (nail polish) was applied to all surfaces of the teeth except for 1 mm adjacent to the restoration margins. The teeth were embedded in acrylic resin blocks (Orthodontic Resin, Dentsply). All specimens were then immersed in 0.5 methylene blue dye solution for 24 hours. The teeth were rinsed in running water, blotted dry, then sectioned longitudinally from facial to lingual surface with a water-cooled diamond wheel saw (Isomet, Buehler, Lake Bluff, IL, USA). Three longitudinal sections were performed: one in the middle and the other two close to the mesial and distal margins of the restoration.[14-15] Dye penetration at the occlusal and gingival margin was examined by two independent evaluators using a stereomicroscope at 20x and scored according to the following criteria: 0=no dye penetration; 1=partial dye penetration along the occlusal or gingival wall; 2=dye penetration along the occlusal or gingival wall, but not including the axial wall; 3=dye penetration to and along the axial wall.[16,17]

RESULTS

Statistical analysis was performed utilizing the Kruskal-Willis one-way ANOVA followed by a Mann-Whitney test. The difference between the occlusal and gingival dye penetration scores for each group was analyzed by the Wilcoxon Signed Rank test. Statistical analysis was performed using the following computer program: Statistical Package for the Social Sciences (SPSS Inc, Chicago, IL, USA).

Dye penetration scores for the occlusal and gingival walls are presented in Table 2. Kruskal-Wallis one-way ANOVA indicated significant differences between groups for occlusal and gingival scores ($\chi^2_{df=3}$ =36, p<0.0001, $\chi^2_{df=3}$ =45.6, p<0.0001, respectively). The Mann-Whitney test was performed to evaluate significant differences of *occlusal scores between groups*. The results demonstrated no significant leakage differences among Xeno III, Prime & Bond NT and Clearfil SE Bond (p>0.05). Conversely, i-Bond had significantly more dye penetration when compared to Xeno III, Prime & Bond NT and Clearfil SE Bond (p<0.0001).

Gingival scores between groups showed some significant differences (p>0.05): i-Bond showed significantly more dye penetration when compared to Xeno III, Prime & Bond NT and Clearfil SE Bond (p<0.0001).

When comparing the *occlusal and gingival scores for each group*, the Wilcoxon Rank test showed no significant difference in dye penetration for Xeno III (p>0.05), Prime & Bond NT (p=0.059) and i-Bond (p=0.083). Clearfil SE Bond yielded more dye penetration at the occlusal wall than at the gingival (p=0.001).

Table 2: *Dye Penetration Scores for the Tested Materials*

	Occlusal				Gingival			
Group	0	1	2	3	0	1	2	3
Xeno III (n=45)	17	23	5	0	15	25	5	0
Prime & Bond NT (n=45)	11	33	1	0	17	26	2	0
i-Bond (n=45)	0	27	16	2	1	26	18	0
Clearfil SE Bond (n=45)	16	25	3	1	23	22	0	0

DISCUSSION

Various techniques have been used to assess dye penetration in microleakage studies. In this study, the evaluation of dye penetration was scored after three faciolingual sections and optical microscopic observation to assess microleakage along the entire length of the preparation interface. Numerous studies utilize a single section through the center of the restoration,[18-20] resulting in *in vitro* microleakage being underestimated.[14-15] A three-dimensional evaluation has also been proposed as an alternative method to evaluate microleakage;[21-22] the entire restoration is removed in an attempt to reveal more extensive dye penetration. This technique is more time consuming and does not allow for good evaluation of dentin tubule leakage.[23] The technique adopted in this study may represent the most appropriate method to assess microleakage.[14-15,24]

The adhesive i-Bond one-step self-etch reported the highest microleakage score both on enamel and dentin, with partial and extensive dye penetration along the occlusal and gingival walls. Conversely, the other one-step self-etch adhesive, Xeno III, reported results very similar to the two-step total-etch adhesive Prime and Bond NT and the two-step mild self-etch adhesive Clearfil SE Bond.

Although a differing performance could be expected between i-Bond and Prime & Bond NT or Clearfil SE Bond, the same difference could not be predicted between i-Bond and Xeno III, since both use a single-step application procedure. According to Van Meerbeek and others,[25] i-Bond and Xeno III have a very similar acidity (pH around 1.5), which should bring about a very similar demineralization pattern.

However, the two "all-in-one" adhesives present some peculiarities. Xeno III is a two part (Liquid A and Liquid B) self-etch adhesive; i-Bond is a one component self-etch adhesive with acidic and resin monomers, solvent and water included in the same bottle. The chemical composition is quite different. One adhesive (Xeno III) uses ethanol as a solvent, the other acetone; the acidic monomers are different: i-Bond utilizes 4-META and Xeno III contains Pyro-EMA. Both systems use UDMA as a resin monomer; however, the balance of water-acidic monomers and resin monomers in self-etch adhesives is paramount in optimizing bond efficacy to dentin.[26]

Xeno III contains nanofillers; previous findings[27-28] reported that the collagen fibril network mostly filters out nanofillers, holding them at the hybrid layer surface, thus acting as an intermediate shock absorber. A reduced microleakage score has been reported when using filled adhesives.[29-30] These differences in chemical composition may have helped to create a thicker, more homogeneous resin layer above the hybrid layer using Xeno III; the authors of this study speculate that it resulted in improved resistance to microleakage. However, King and others[31] reported that the improved performance of both Xeno III and i-Bond can be achieved by converting the one step to a two step self-etch adhesive through the adjunctive application of a hydrophobic resin layer. The resin layer is applied and light cured over the self-etch adhesives. This resin coat helps to reduce the amount of hydrophilic and acidic resin component in the bonded dentin interface, rendering these self-etch adhesives less permeable.

Clearfil SE Bond is a mild two-step self-etch adhesive with a pH very close to 2[25]. It was not surprising to see more extensive leakage on enamel than dentin with this product; it is well known that the bonding effectiveness of mild two-step self-etch adhesive to enamel is questionable. Some laboratory studies reported equal or reduced enamel bonding effectiveness as compared to conventional phosphoric acid etching.[6,32-33] However, in this study, there was no significant difference when comparing the enamel microleakage scores of Clearfil SE Bond and Prime & Bond NT two-step total-etch adhesives.

Mild self-etch adhesives produce hybrid layers thinner than total-etch systems. As dentin demineralization is less pronounced, smear plugs occlude the orifice of the dentinal tubules, which are partially infiltrated by resin; a reduced resin tag formation occurs with these systems.[32,34] Despite the limited thickness of HL, Clearfil SE Bond has been reported to result in very high dentin bond strengths comparable to or even higher than the ones obtained with total-etch adhesives.[25,35] These findings corroborate the results of this study: Clearfil SE Bond reported minimal dye penetration in dentin. Noteworthy, collagen fibrils within the hybrid layer are not completely deprived of hydroxyapatite; it was hypothesized that the residual hydroxyapatite may serve as a receptor for additional intermolecular interaction with specific monomers of the mild self-etch adhesive.[1] It was suggested that the bonding effectiveness of mild self-etch adhesives may result from a combined micromechanical and chemical interaction with tooth substrate;[25,36] the chemical component may be able to compensate for the reduced bonding effectiveness from decreased micromechanical interlocking.

Interestingly, Prime & Bond NT reported enamel and dentin microleakage scores similar to both Clearfil SE Bond and Xeno III; decreased dye penetration was expected for Prime & Bond NT, at least at the enamel cavosurface margins. The authors of this study hypothesized that the hybrid layer created by Prime & Bond NT may be more susceptible to a hydrolytic process than the hybrid layer that forms as an onsequence of the application of self-etch adhesives. Koshiro and others[37-38] reported that the bonding interface, using a mild self-etch adhesive, was more stable over time than the bonding interface of a total-etch adhesive. The combination of mild self-etch adhesives, micromechanical and chemical bonding may increase the longevity of the restorations, as the chemical interaction may result in bonds that better resist hydrolytic degradation (nanoleakage).[1,4,25]

This research study adopted a combination of two commonly used aging processes to simulate the degradation of bond over time in the oral cavity: aging by storage and aging by thermocycling. The efficacy of thermocycling on microleakage as a simulation of clinical aging has been the subject of controversy among researchers.[39-40] De Munck and others reported that the effect of thermocycling and water storage on microleakage is minimal compared with its effect in bond strength tests.[4] Noteworthy was the resulting enamel and dentin microleakage score for Prime & Bond NT. It was higher than that reported in other research studies where thermocycling was the only artificial aging method adopted.[30,41] Although a single section through the center of the restoration was adopted in these research studies, it may be speculated that the combination of the two aging processes can increase the effect on artificial aging, thus increasing microleakage. Further research should focus on the effect of these combined artificial aging methods on microleakage through the use of proper controls; comparison of three- or two-step total-etch vs self-etch adhesives may be required with and without the effect of differing artificial aging methods.

As manufacturers launch new "all-in-one" self-etch adhesives before the conclusion of independent ongoing studies, efforts toward future research should be directed to assess the quality and reliability of these materials through both laboratory and clinical evaluations. These steps should be taken before their introduction to the market

CONCLUSIONS

The results of this *in vitro* study rebut the common belief that simpler, speedier, more user-friendly adhesives always reduce technique sensitivity. Although no over-drying or over-etching of dentin can occur with the use of self-etch adhesives, some systems, especially "all-in-ones," demonstrated sub-par performance when compared to total-etch adhesives; recent research supports this finding.[42]

The first null hypothesis was partially satisfied: i-Bond one–step self-etch adhesive reported higher enamel and dentin microleakage scores than two-step self-etch and two-step total-etch adhesives; conversely, Xeno III one–step self-etch adhesive performed as well as the other two adhesives evaluated.

All the adhesives tested performed as well in enamel as in dentin, with the exception of Clearfil SE Bond. Clearfil reported higher microleakage scores in enamel than in dentin.

(Received 9 April 2006)

References

1. Van Meerbeek B, Vargas M, Inoue S, Yoshida Y, Peumans M, Lambrechts P & Vanherle G (2001) Adhesives and cements to promote preservation dentistry *Operative Dentistry* **(Supplement 6)** 119-144.
2. Blunck U (2000) Adhesives: Principles and state of the art. In JF Roulet M & Degrange *Adhesion the Silent Revolution in Dentistry* Quintessence Publishing Co Chicago IL.
3. Swift EJ Jr, Perdigão J, Wilder AD, Heymann HO, Sturdevant JR & Bayne SC (2001) Clinical evaluation of two one-bottle dentin adhesives at three years *Journal of the American Dental Association* **132** 1117-1123.
4. De Munck J, Van Landuyt K, Peumans M, Poitevin A, Lambrechts P, Braem M & Van Meerbeek B (2005). A critical review of the durability of adhesion to tooth tissue: Methods and results *Journal of Dental Research* **84** 118-132.
5. Tay FR & Pashley DH (2001) Aggressiveness of contemporary self-etching systems. I: Depth of penetration beyond dentin smear layers *Dental Materials* **17** 296-308.
6. Pashley DH & Tay FR (2001) Aggressiveness of contemporary self-etching systems. II: Etching effects on unground enamel *Dental Materials* **17** 430-444.
7. Christensen GJ (2001) Self-etching primers are here *Journal of the American Dental Association* **132** 1041-1043.
8. Senawongse P, Harnirattisai C, Shimada Y & Tagami J (2004) Effective bond strength of current adhesive systems on deciduous and permanent dentin *Operative Dentistry* **29** 196-202.
9. Sattabanasuk V, Shimada Y & Tagami J (2004) The bond of resin to different dentin surface characteristics *Operative Dentistry* **29** 333-341.
10. Perdigão J & Geraldeli S (2003) Bonding characteristics of self-etching adhesives to intact versus prepared enamel *Journal of Esthetic & Restorative Dentistry* **15** 32-42.
11. Tay FR, Pashley DH, Suh BI, Carvalho RM & Itthagarun A (2002) Single-step adhesives are permeable membranes *Journal of Dentistry* **30** 371-382.
12. Tay FR, Pashley DH, Suh BI, Carvalho RM & Miller M (2004) Single-step, self-etch adhesives behave as permeable membranes after polymerization Part I. Bond strength and morphologic evidence *American Journal of Dentistry* **17** 315-322.
13. Tay FR, Pashley DH, García-Godoy F & Yiu CK (2004). Single-step, self-etch adhesives behave as permeable membranes after polymerization. Part II. Silver tracer penetration evidence *American Journal of Dentistry* **17** 315-322.
14. Ferrari M & García-Godoy F (2002) Sealing ability of new generation adhesive-restorative materials placed on vital teeth *American Journal of Dentistry* **15** 117-128.
15. Raskin A, Tassery H, D'Hoore W, Gonthier S, Vreven J, Degrange M & Dejou J (2003) Influence of the number of sections on reliability of *in vitro* microleakage evaluation *American Journal of Dentistry* **16** 207-210.
16. Toledano M, Osorio E, Osorio R & García-Godoy F (1999) Microleakage of Class V resin-modified glass ionomer compomer restorations *Journal of Prosthetic Dentistry* **81** 610-615.
17. Toledano M, Perdigão J, Osorio E & Osorio R (2000) Effect of dentin deproteinization on microleakage of Class V composite restorations *Operative Dentistry* **25** 497-504.
18. Hakimeh S, Vaidyanathan J, Houpt ML, Vaidyanathan TK & Von Hagen S (2000) Microleakage of compomer Class V restorations: Effect of load cycling, thermal cycling, and cavity shape *Journal of Prosthetic Dentistry* **83** 194-203.
19. Jang KT, Chung DH, Shin D & García-Godoy F (2001) Effect of eccentric load cycling on microleakage of Class V flowable and packable composite resin restorations *Operative Dentistry* **26** 603-608.
20. Deliperi S, Bardwell DN, Papathanasiou A, Kastali S & García-Godoy F (2004) Microleakage of a microhybrid composite using three different adhesive placement techniques *Journal of Adhesive Dentistry* **6** 135-140.
21. Mixson JM, Eick JD, Chappell RP, Tira DE & Moore DL (1991) Comparison of two surface and multi surface scoring methodologies for *in vitro* microleakage studies *Dental Materials* **7** 191-196.

22. Gwinnett JA, Tay FR, Pang KM & Wei SHY (1995) Comparison of three methods of critical evaluation of microleakage along restorative interfaces *Journal of Prosthetic Dentistry* **74** 575-585.

23. Hilton TJ, Schwartz RS & Ferracane JL (1997) Microleakage of four Class II resin composite insertion techniques at intraoral temperature *Quintessence International* **28** 135-144.

24. Vandewalle KS, Ferracane JL, Hilton TJ, Erickson RL & Sakaguchi RL (2004) Effect of energy density on properties and marginal integrity of posterior resin composite restorations *Dental Materials* **20** 96-106.

25. Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, Van Landuyt K, Lambrechts P & Vanherle G (2003) Buonocore Memorial Lecture. Adhesion to enamel and dentin: Current status and future challenges *Operative Dentistry* **28** 215-235.

26. Hiraishi N, Nishiyama N, Ikemura K, Yau JY, King NM, Tagami J, Pashley DH & Tay FR (2005). Water concentration in self-etching primers affects their aggressiveness and bonding efficacy to dentin *Journal of Dental Research* **84** 653-658.

27. Tay FR, Moulding KM & Pashley DH (1999) Distribution of nanofillers from a simplified-step adhesive in acid conditioned dentin *Journal of Adhesive Dentistry* **1** 103-117.

28. Inoue S, Vargas M, Abe Y, Yoshida Y, Lambrechts P, Vanherle G, Sano H & Van Meerbeek B (2001) Microtensile bond strength of eleven contemporary adhesives to dentin *Journal of Adhesive Dentistry* **3** 237-245.

29. Fortin D, Swift EJ Jr, Denehey GE et al (1994) Bond strength and microleakage of current dentin adhesives *Dental Materials* **10** 253-258.

30. Deliperi S, Bardwell DN, Papathanasiou A & Perry R (2003) Microleakage of resin-based liner materials and condensable composites using filled and unfilled adhesives *American Journal of Dentistry* **16** 351-355.

31. King NM, Tay FR, Pashley DH, Hashimoto M, Ito S, Brackett WW, García-Godoy F & Sunico M (2005) Conversion of one-step to two-step self-etch adhesives for improved efficacy and extended application *American Journal of Dentistry* **18** 126-134.

32. Hannig M, Reinhardt KJ & Bott B (1999) Self-etching primers vs phosphoric acid: An alternative concept for composite-to-enamel bonding *Operative Dentistry* **24** 172-180.

33. Kanemura N, Sano H & Tagami J (1999) Tensile bond strength and SEM evaluation of ground and intact enamel surfaces *Journal of Dentistry* **27** 523-530.

34. Prati C, Chersoni S, Mongiorgi R & Pashley DH (1998) Resin-infiltrated dentin layer formation of new bonding systems *Operative Dentistry* **23** 185-194.

35. Cardoso PE, Sadek FT, Goracci C & Ferrari M (2002) Adhesion testing with the microtensile method: Effects of dental substrate and adhesive system on bond strength measurement *Journal of Adhesive Dentistry* **4** 291-297.

36. Yoshida Y, Nagakane K, Fukuda R, Nakayama Y, Okazaki M, Shintani H, Inoue S, Tagawa Y, Suzuki K, De Munck J & Van Meerbeek B (2004) Comparative study on adhesive performance of functional monomers *Journal of Dental Research* **83** 454-458.

37. Koshiro K, Inoue S, Tanaka T, Koase K, Fujita M, Hashimoto M & Sano H (2004) *In vivo* degradation of resin-dentin bonds produced by a self- vs a total-etch adhesive system *European Journal of Oral Science* **112** 368-375.

38. Koshiro K, Inoue S, Sano H, De Munck J & Van Meerbeek B (2005) *In vivo* degradation of resin-dentin bonds produced by a self-etch and an etch-and-rinse adhesive *European Journal of Oral Science* **113** 341-348.

39. Doerr CL, Hilton TJ & Hermesch CB (1996) Effect of thermocycling on the microleakage of conventional and resin modified glass ionomer *American Journal of Dentistry* **9** 19-21.

40. Yap AUJ (1997). Effects of storage, thermal and load cycling on a new reinforced glass-ionomer cement *Journal of Oral Rehabilitation* **25** 40-44.

41. Deliperi S, Bardwell DN, Wegley C & Congiu MD (2006) *In vitro* evaluation of Giomers microleakage after exposure to 33% hydrogen peroxide: Self-etch vs total-etch adhesives *Operative Dentistry* **31** 227-232.

42. Söderholm KM, Guelmann M & Bimstein E (2005) Shear bond strength of one 4th and two 7th generation bonding agents when used by operators with different bonding experience *Journal of Adhesive Dentistry* **7** 57-64.