©Operative Dentistry, 2009, **34-5,** 571-577

Effect of Double Layering and Prolonged Application Time on MTBS of Water/Ethanol-based Self-etch Adhesives to Dentin

MC Erhardt • R Osorio • J Pisani-Proenca
FS Aguilera • E Osorio • L Breschi • M Toledano

Clinical Relevance

Problems related to the chemical formulation and/or chemical dispersion of hydrophilic/hydrophobic components in one-step self-etch adhesives limit their efficacy and are not easily solved by changing the different bonding application parameters.

Maria Carolina Erhardt, DDS, MS, PhD, Campus de Cartuja s/n, School of Dentistry, Granada, Spain

Raquel Osorio, LDS, PhD, University of Granada, Dental Materials, Campus Universitario de Cartuja s/n, School of Dentistry, Colegio Maximo, Granada, Spain

Jatyr Pisani-Proenca, LDS, Campus de Cartuja s/n, Granada, Spain

Fatima S Aguilera, DDS, PhD, University of Granada, Dental Materials, Campus de Cartuja s/n, School of Dentistry, Colegio Máximo, Granada, Spain

Estrella Osorio, DDS, PhD, University of Granada, Dental Materials, Campus de Cartuja s/n, Granada, Spain

Lorenzo Breschi, DDS, PhD, University of Trieste, Biomedicine, Trieste & EM-CNR, Bologna, Italy

*Manuel Toledano, MD, BDS, PhD, University of Granada, Dental Materials, Campus Universitario de Cartuja s/n, School of Dentistry, Colegio Máximo, Granada, Spain

*Reprint request: Granada, Granada E-18071, Spain; e-mail: toledano@ugr.es

DOI: 10.2341/08-060-L

SUMMARY

One way of possibly improving bond strength is by changing the application mode of self-etch adhesives. The current study evaluated the resin-dentin microtensile bond strength (MTBS) promoted by two- and one-step self-etching adhesives after different bonding application procedures. Flat dentin surfaces from extracted human molars were bonded: 1) according to the manufacturers' instructions, 2) duplicating the number of adhesive coats and 3) doubling the application time of the acidic primers. Two-step (Clearfil SE Bond/SEB and Resulcin AquaPrime/RE) and one-step (Etch & Prime 3.0/EP and One-Up Bond F/OUB) self-etch adhesives were used. Resin-dentin beams were tested in tension at 0.5 mm/minute. Selected debonded beams were observed under scanning electron microscopy (SEM). MTBS data were analyzed by ANOVA and multiple comparison tests (*p*<0.05).

The highest MTBS was always attained with SEB, regardless of the bonding procedure. RE, EP and OUB showed similar MTBS when bonded as per the manufacturers' instructions. The MTBS of OUB increased after doubling the application time and duplicating the adhesive coats. The two-step self-etch adhesives were insensible to changes in bonding application procedures. Attempts to improve the bonding performance of water/ethanol-based self-etching systems by using different bonding application parameters were system-specific and only effectively detected in one-step adhesive systems.

INTRODUCTION

The use of self-etching agents eliminates the conditioning, rinsing and drying steps that have been shown to be both critical and difficult to standardize in operative conditions, because of the instability of the demineralized dentin matrix.[1-2] Two-step self-etching primers combine the acid and the primer in one solution to form an acidic monomer, followed by the application of a resin monomer.[3] One-step self-etching adhesives were developed in order to shorten the bonding procedure and reduce the sensitivity of the technique,[4] since all of the components are blended in one solution.[1]

A poor bonding performance has been reported for some of these simplified adhesives,[5-6] the main reasons being: 1) the presence of highly hydrophilic monomers that are sensitive to water sorption from the underlying dentin,[7] increasing hybrid layer permeability and nanoleakage;[4,8-9] 2) a differential infiltration gradient through the dentin related to differences in the molecular weight of the adhesive system compounds;[10] 3) a high concentration of protic solvents and dissolved molecular oxygen within the adhesive layer as a result of poor evaporation;[11-12] 4) the limited thickness of the adhesive layer,[12] which may also magnify the oxygen inhibition effect on adhesive polymerization[13] and 5) their low degree of cure.[11]

Interactions of the self-etch agents with dentin are limited by many factors from both the substrate[14] and the adhesive itself.[6,9,15] Optimal resin infiltration is important to achieving adequate bond strengths,[9,16-17] as effective resin-dentin bonds may only be formed if primers and resins are able to penetrate through the smear layer and interact with the underlying dentin.[18-20] The infiltration of the adhesive blend, solvent/water evaporation and thickness of the adhesive layer are directly related to chemical and rheological properties,[11-12,21-22] but may be influenced by the application mode.[23] It has been hypothesized that more uniform adhesive infiltration and greater solvent/water evaporation would be achieved when bonding with double layering[13,24] or by a prolonged application time of the primers/adhesives onto the dentin surface.[22,25-26]

Therefore, the current study determined the effect of duplicating the coats or extending the application time of self-etch bonding systems on dentin microtensile bond strength (MTBS). The null hypothesis tested was: 1) there is no difference in attained MTBS to dentin between the tested adhesives and 2) duplicating the number of adhesive layers or doubling their application time does not affect MTBS to dentin of any of the self-etch adhesives tested.

METHODS AND MATERIALS

Thirty-six extracted caries-free human third molars were stored at 4°C in 1% thymol solution for up to one month. The occlusal enamel was ground under running water with 180-grit SiC papers to provide uniform dentin surfaces with clinically relevant smear layers for bonding.[27]

Table 1 displays the mode of application and components of the self-etch adhesive systems used in the experiment: Clearfil SE Bond (Kuraray Co Ltd, Osaka, Japan), Resulcin AquaPrime (Merz Dental, Lütjenburg, Germany), Etch & Prime 3.0 (Degussa AG, Hanau, Germany) and One-Up Bond F (Tokuyama Europe GmbH, Dusseldorf, Germany). The surfaces were rinsed for one minute with distilled water and gently air-dried before adhesive application. The teeth were randomly divided into 12 equal groups to be bonded with the four different self-etch adhesives as follows: 1) according to the manufacturers' instructions; 2) the application of two layers of primer (for the one-step systems, after light-curing the first layer, the adhesive was reapplied and light-cured) and 3) doubling the primer/adhesive application time.

Resin build-ups, each 6 mm in height, were constructed incrementally (2.0 mm) with Tetric Ceram (Ivoclar Vivadent, Schäan, Liechtenstein) light-cured hybrid resin composite. Each layer of the composite was light activated for 40 seconds with a Translux EC halogen light-curing unit (Kulzer GmbH, Wehrheim, Germany). Light intensity was monitored with a radiometer (Model 100, Kerr Demetron, Danbury, CT, USA) to be at least 600 mW/cm^2. After 24 hours of water storage at 37°C, the bonded teeth were vertically sectioned into serial slabs that were further sectioned into 1.0 mm^2 composite-dentin beams.

The beams were attached to a modified Bencor Multi-T testing apparatus (Danville Engineering Co, San Ramon, CA, USA) with a cyanoacrylate adhesive (Zapit, Dental Ventures of America Inc, Corona, CA, USA) and tensioned in a universal testing machine (Instron Inc, Canton, MA, USA) at a crosshead speed of 0.5 mm/minute. The fractured beams were carefully removed from the apparatus and the cross-sectional

Table 1: *Main Components and Application Mode of Bonding Agents Used in the Experimental Groups*

Self-etching Category	Components	Principal Ingredients (according to the manufacturers)	Mode/Steps of Application
2-step	*Clearfil SE Bond (pH= 2.1)*		
	Primer	10-MDP; HEMA; hydrophilic dimethacrylate; dl-camphorquinone; N,N-diethanol-p-touidine; water.	Apply Primer for 20 seconds. Mild air stream.
	Bond	10-MDP; Bis-GMA; HEMA; hydrophobic dimethacrylate; di-camphorquinone; N,N-diethanol-p-toluidine; silanated colloidal silica.	Apply Bond. Gentle air stream. Light cure for 10 seconds.
	Resulcin AquaPrime + Monobond (pH= 1)		
	AquaPrime	2-methacryloyloxyethyl-dihydrogen-phosphate.	Mix AquaPrime with water (1:1). Scrub into the dentin surface for 30 seconds. Gently air dry.
	Monobond	Bis-GMA, TEGDMA, polymethacryl-oligomaleic acid.	Apply Monobond. Air blow gently. Light cure for 20 seconds.
One-step	*Etch & Prime 3.0 (pH= 0.76)*		
	Universal	HEMA, water, ethanol, stabilizer.	Mix Universal and Catalyst. Apply for 30 seconds. Air blow gently. Light cure for 10 seconds.
	Catalyst	HEMA, initiators, stabilizers, tetra-methacryloyloxyethylpyrophosphate (HEMA-phosphate)	
	One-Up Bond F (pH= 1.2)		
	Agent A	Phosphoric monomer, MAC-10, multifunctional methacrylic monomer, co-initiator.	Mix bonding A & B (1:1). Apply mixed material for 20 seconds. Gently air stream. Light cure for 20 seconds.
	Agent B	HEMA, water, fluoroaluminosilicate microfiller, dye-sensitizer, borate derivative catalyst.	

Abbreviations: Bis-GMA: bis-phenol A diglycidylmethacrylate; TEGDMA: triethylene glycol-dimethacrylate; MAC-10: Methacryloyloxyalkyl acid phosphate; 10-MDP: 10-methacryloxydecyl dihydrogen phosphate; HEMA: 2-hydroxyethyl methacrylate.

area at the site of failure measured to the nearest 0.01 mm with a pair of digital calipers (Sylvae Ultra-Call LI, Fowler Inc, Newton, MA, USA). The fractured specimens were examined with a stereomicroscope (Olympus/DeTrey, Konstanz, Germany) at 40x magnification to determine the mode of failure. The failure modes were classified as adhesive, cohesive or mixed. Representative specimens of each group were kept for 48 hours in a desiccator (Sample Dry Keeper Simulate Corp, Tokyo, Japan), then mounted on aluminum stubs with carbon cement. The specimens were then sputter-coated with pure gold by means of a sputter-coating Unit E500 (Polaron Equipment Ltd, Watford, England) and observed with a scanning electron microscope (SEM) (1430 VP, LEO Electron Microscopy Ltd, Cambridge, UK) at an accelerating voltage of 20 kV, in order to observe the microscopic fracture patterns and morphology of the debonded interfaces.

A two-way analysis of variance (ANOVA) was used to examine the effect of the variable adhesive system and bonding procedure on the MTBS. Multiple comparisons were performed using the Student-Newman-Keuls test. The statistical significance was set at α=0.05.

The pH of the bonding systems was assessed using pH indicator strips (Duotest, Macherey-Nagel, Düren, Germany).

RESULTS

The pH values of the primers of each adhesive system are shown in Table 1. The mean MTBS values obtained in each group are summarized in Table 2. Bond strength to dentin was affected by the adhesive system (p<0.001) and bonding procedure (p<0.0001). The interactions of these two factors were also significant (p<0.0001). The power of the multiple ANOVA (R^2) was around 63%.

Once bonded per the manufacturers' instructions, the highest MTBS was attained by SEB, and the rest of the adhesives that were tested performed similarly. Regarding bonding procedure, the highest MTBS was always attained with SEB. MTBS attained by two-step self-etch adhesives (SEB and RE) was not affected by

Table 2: *Mean MTBS (MPa) and Standard Deviation (SD) of the Four Self-etch Adhesives Bonded After Different Application Procedures*

Adhesives	Bonding Parameter					
	Manufacturers	n	Double Layer	n	Double Time	n
SEB	44.0 (12.2)^{A,1}	32	34.7 (8.3)^{A,1}	32	39.0 (8.8)^{A,1}	34
RE	11.5 (4.9)^{B,1}	31	8.6 (2.8)^{C,1}	30	12.6 (3.6)^{C,1}	28
EP	17.0 (7.8)^{B,1}	29	9.0 (2.9)^{C,2}	30	22.1 (9.8)^{B,1}	31
OUB	15.5 (5.6)^{B,2}	30	20.0 (7.5)^{B,1}	31	21.8 (7.6)^{B,1}	32

Results of post-hoc multiple-comparison tests are indicated in the superscripts. For each column, groups labeled with the same letter superscripts are not significantly different (p>0.05). For each row, groups labeled with the same number are not significantly different (p>0.05).
Beams that failed prematurely were not included in the statistical analysis. The actual number of composite-dentin beams tested for each subgroup (n) is included.

Table 3: *Distribution (as percentage) of Failure Modes: A—adhesive, M—mixed*

	Bonding Parameter					
	Manufacturers		Double Layer		Double Time	
Adhesives/Fracture Mode	A	M	A	M	A	M
SEB	37	63	41	59	32	68
RE	55	45	73	27	61	39
EP	57	43	68	32	63	37
OUB	62	38	46	54	38	62

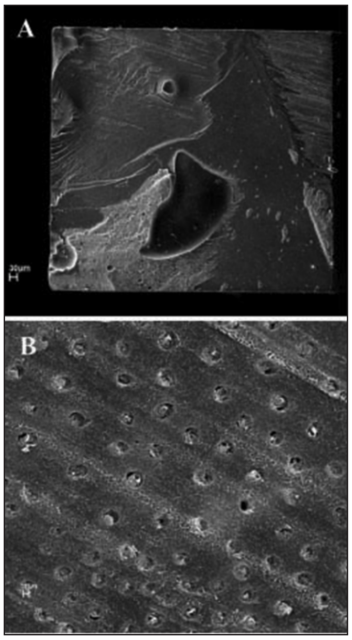

Figure 1. *SEM images of the debonded dentin surface of an SEB specimen applied as per the manufacturers' instructions. (A) A mixed failure, in which a large area of remaining adhesive resin may be observed (bar=30 μm). (B) At a higher magnification, entrances of the dentinal tubules may be observed. They are not enlarged and are occluded by hybridized resin plugs. Zones of cohesive fracture within the hybridized smear layer are shown (bar=10 μm).*

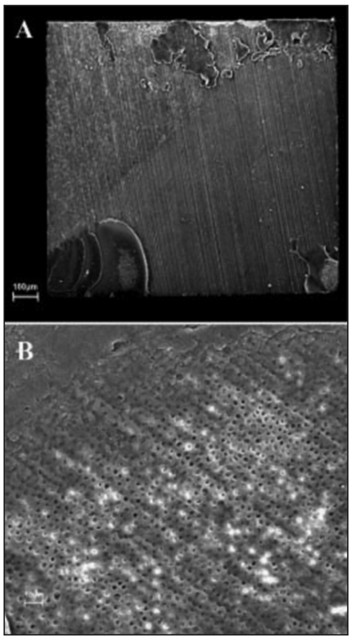

Figure 2. *SEM images of the debonded dentin site of an RE specimen after a double layering application procedure. (A) An adhesive failure is shown, which mainly occurred below the hybrid complex, and a few areas of adhesive resin remain at the periphery (bar=100 μm). (B) At a higher magnification, areas of fracture exposing the underlying dentin are shown, dentinal tubules are enlarged and few resin tags were observed (bar=10 μm).*

double layering or extended application time. However, the efficacy of the one-step adhesive systems was affected by these different application procedures. Duplication of the adhesive coats lowered the MTBS values of EP. Duplicating the layering and doubling the application time significantly increased bond strength values when OUB was tested.

Table 3 displays the percentage of failure modes of the debonded specimens. Most of the failures were adhesive, except for SEB (regardless of the bonding technique), and when OUB was used, either when doubling the adhesive layers or extending the application time. In both cases, a higher percentage of mixed failures was obtained. No cohesive failures were observed.

SEM examinations of debonded dentin surfaces after MTBS testing are shown in Figures 1 to 4. Images from SEB specimens showed predominately mixed failures (Figure 1A). At high magnification, cohesive failures within the hybridized smear layer are observed (Figure 1B). For the RE groups (Figure 2), failures usually occurred at the bottom of the hybridized complex (scratches from previous dentin grinding are clearly observed) and tubule entrances are enlarged and completely devoid of resin tags. In general, specimens bonded with one-step self-etch systems presented adhesive failures that were found at the bottom of the hybridized complex, usually associated with low bond strength data. When EP specimens were examined (Figure 3), opened and enlarged, dentin tubule entrances without resin tags were encountered. A mixed failure could be observed after prolonged application time in OUB-bonded specimens (Figure 4). Small areas exposing resin-covered dentin were detected and a few tubules were visible.

DISCUSSION

The two-step self-etching system SEB always revealed the highest MTBS, irrespective of the bond

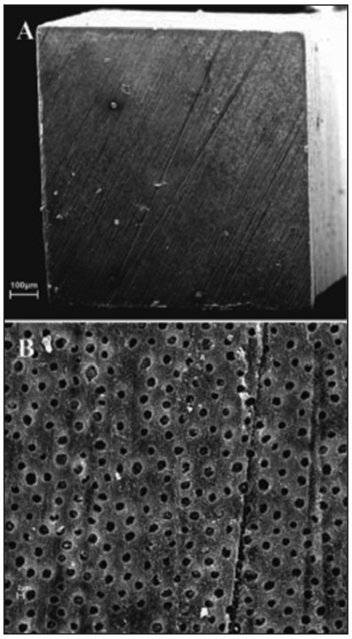

Figure 3. *SEM observations of the debonded surface along the dentin side of a specimen bonded with EP as per the manufacturers' instructions. (A) An adhesive failure at the bottom of the smear layer may be observed (bar=100 µm). (B) At a higher magnification, the dentinal tubules are enlarged with no resin tags. Intertubular dentin appeared porous as after being etched and improperly infiltrated by resin (bar=2 µm).*

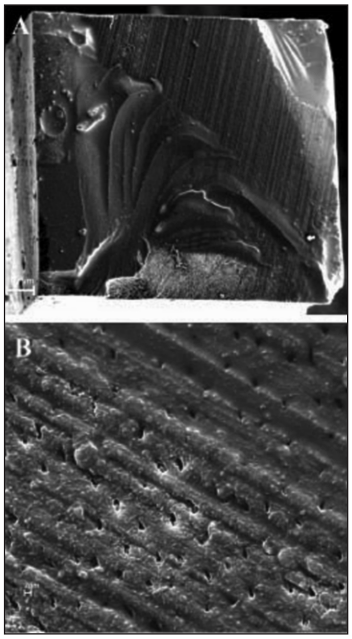

Figure 4. *SEM images of the debonded interface on the dentin side when OUB was used with a prolonged application time. (A) A mixed failure is presented (bar=100 µm). (B) At a higher magnification, a fracture within the hybridized complex is shown; some tubule entrances are visible, dentin appears covered with resin (bar=2 µm).*

ing application procedure. The higher performance of this adhesive system has been previously reported[5,9,20] and may be related to: 1) use of a mild self-etching primer (pH=2.1) that leads to a negligible dissolution of the smear plugs,[9,21,28] facilitating penetration, entanglement and polymerization of monomers with the underlying dentin to form accurate hybrid layers;[1,5,6] 2) incorporation of the functional monomer 10-MDP in combination with HEMA, which improves the wetting of the tooth surface and chelates to calcium ions of dentin,[6,22] creating hardly soluble calcium-salts[21] and 3) the presence of photoinitiators in both components (primer and bond), which increases the photo-polymerization efficacy of the monomers and facilitates solvent evaporation.[11,29] A high percentage of mixed failures was obtained (Table 3), probably due to SEB's improved dentin hybridization, since a mild dentin demineralization pattern that did not affect the peritubular dentin and permitted hybridized resin plugs formation was encountered (Figure 1).

RE is a two-step self-etch adhesive that also showed stable performance regardless of the applied bonding procedure. However, the bond strength attained by RE was lower than that of SEB. "Strong" self-etch adhesives (pH≤1, eg, RE) performed poorly, compared with "mild" self-etch adhesives (pH≤1, eg SEB).[9,28] When aggressive versions (pH≤1) of simplified adhesives are applied, the smear plugs are dissolved and the tubules opened,[7] facilitating phase separation and dilution of the subsequent applied resin.[10] Water entrapment also adversely affects adhesive polymerization.[7] Diffusion of Resulcin Monobond (which is mainly Bis-GMA and TEGDMA-based resins) on this demineralized dentin surface with enlarged tubules (Figure 2B) may be strongly limited, as these resin monomers have a high molecular weight and low affinity to water-rich substrates. This results in the formation of a superficial hydrophobic layer that may offer rigidity but exhibits low monomer conversion.[10,30] The existence of a partially etched but poorly infiltrated dentin zone beneath the hybridized smear layer may lead to the formation of a weak potential site for bonding failure prone to degradation.[31-32] Self-etching systems are very complicated chemistries. Even though most adhesive systems contain the same components, they may differ significantly, considering the proportional amount of ingredients, such as resin, initiator, inhibitor, solvent and filler particles.[22,28] As a consequence, particular shortcomings related to the specific composition of the tested adhesive systems might be considered as explaining the different bonding effectiveness obtained with these adhesives.

Extending the priming time of RE or the supplementary application coat of the RE primer did not increase resin-dentin MTBS. These procedures are not able to overcome the main problem, which is the poor infiltration and polymerization of hydrophobic resins into this water-rich substrate. Moreover, increasing the concentration of 2-methacryloyloxyethyl-dihydrogen-phosphate at the tooth surface may result in dentin overetching and/or a decrease in adhesive layer polymerization related to an increase in the oxygen inhibition effect.[33-34]

The one-step systems OUB and EP consistently obtained lower MTBS values when compared to the two-step system SEB.[5-6,9,20] The lower MTBS of these one-step self-etch bonding agents may be associated with phase separation and a competitive and differential diffusion of hydrophilic and hydrophobic monomers into dentin, resulting in heterogeneous hybrid layers.[7,30] These complex blends contain high concentrations of solvents that are not properly evaporated,[8,30,35] affecting resin polymerization and lowering the strength of the resin-dentin bonds.[7,11]

When two coats or double application time were used with OUB, the MTBS was higher than when the manufacturers' instructions were followed. Increasing the application time may facilitate solvent evaporation and adhesive infiltration.[23,25-26] Since defective bond formation can also be related to oxygen inhibition of the thin adhesive layer, multi-layering also has a beneficial effect, creating a thicker adhesive layer and/or improving resin infiltration and covering decalcified dentin.[36-38] It is also possible that the application of a supplementary coat of OUB or doubling the application time helped to overcome the differential infiltration effect that is produced when hydrophilic and hydrophobic resins are placed simultaneously in a water-rich substrate.[7,10,30] As a result of these changes in the bonding procedure, a more homogeneous, thick adhesive layer can be obtained. How successfully these bonding protocols are in the long run remains to be determined.

Increasing the application time did not improve the dentin MTBS of EP. When water is added as a solvent to comonomer-ethanol mixtures, an increased retention of ethanol and water is produced, since both can hydrogen bond to the monomers.[39] In these cases, air-drying[11,39] or a prolonged application time cannot induce greater solvent evaporation. Duplication of the number of coats lowered the MTBS of EP, as it was not enough to compensate for the lack of an additional solvent-free hydrophobic resin-layer application.[11,35] Since this adhesive does not contain any hydrophobic dimethacrylates in its formulation (Table 1), it is expected that the application of an additional HEMA layer would also facilitate water retention at the adhesive layer, due to the lowering of vapor pressure of the water by HEMA.[1]

The null hypothesis that there is no difference in the resin-dentin MTBS of the tested self-etching adhesive systems when using different bonding procedures must be rejected. One-step self-etch adhesives, which were introduced and marketed as user-friendly bonding agents, are highly technique-sensitive. Problems relating to the chemical formulation and/or molecular dispersion of hydrophilic/hydrophobic components seem to limit the efficacy of these simplified adhesives.

CONCLUSIONS

Within the limits of the current study, it may be concluded that:

1. Bonding of the water/ethanol-based two-step self-etch adhesives Clearfil SE Bond and Resulcin Monobond is less technique-sensitive. The bond strengths attained with two-step adhesives seem to be higher when using milder (pH>1) formulations that possess an additional mechanism of ionic bonding (10-MDP) to dentin.
2. Bonding to dentin with the one-step adhesives Etch and Prime 3.0 and One-Up Bond F is less reliable and technique-sensitive. Bond strength improvements may be achieved by selected changes in bonding parameters, but the results are system-specific.

Acknowledgements

This research project was supported by grants CICYT/FEDER MAT2008-02347\MAT and grants from JA—P07-CTS-2568 and P08-CTS-3944.

(Received 23 October 2008)

References

1. Inoue S, Van Meerbeek B, Vargas M, Yoshida Y, Lambrechts P & Vanherle G (2000) Adhesion mechanism of self-etching adhesives In: Tagami J, Toledano M & Prati C (eds) *Advanced Adhesive Dentistry* Italia Cirimido 131-148.
2. Tay FR, Gwinnett AJ & Pang KM (1996) Resin permeation into acid-conditioned, moist, and dry dentin: A paradigm using water-free adhesive primers *Journal of Dental Research* **75(4)** 1034-1044.
3. Watanabe I, Nakabayashi N & Pashley DH (1994) Bonding to ground dentin by a Phenyl-P self-etching primer *Journal of Dental Research* **73(6)** 1212-1220.
4. Tay FR, Pashley DH & Yoshiyama M (2002) Two modes of nanoleakage expression in single-step adhesives *Journal of Dental Research* **81(7)** 472-476.
5. Osorio R, Toledano M, De Leonardi G & Tay F (2003) Microleakage and interfacial morphology of self-etching adhesives in Class V resin composite restorations *Journal of Biomedical Material Research B Applied Biomaterials* **66(1)** 399-409.
6. Toledano M, Osorio R, de Leonardi G, Rosales-Leal JI, Ceballos L & Cabrerizo-Vilchez MA (2001) Influence of self-etching primer on the resin adhesion to enamel and dentin *American Journal of Dentistry* **14(1)** 205-210.
7. Wang Y & Spencer P (2005) Continuing etching of an all-in-one adhesive in wet dentin tubules *Journal of Dental Research* **84(4)** 350-354.
8. Tay FR & Pashley DH (2003) Water treeing—a potential mechanism for degradation of dentin adhesives *American Journal of Dentistry* **16(1)** 6-12.
9. Carvalho RM, Chersoni S, Frankenberger R, Pashley DH, Prati C & Tay FR (2005) A challenge to the conventional wisdom that simultaneous etching and resin infiltration always occurs in self-etch adhesives *Biomaterials* **26(9)** 1035-1042.
10. Eliades G, Vougiouklakis G & Palaghias G (2001) Heterogeneous distribution of single-bottle adhesive monomers in the resin-dentin inter-diffusion zone *Dental Materials* **17(4)** 277-283.
11. Nunes TG, Garcia FC, Osorio R, Carvalho R & Toledano M (2006) Polymerization efficacy of simplified adhesive systems studied by NMR and MRI techniques *Dental Materials* **22(10)** 963-972.
12. 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(5)** 315-322.

13. Pashley EL, Agee KA, Pashley DH & Tay FR (2002) Effects of one versus two applications of an unfilled, all-in-one adhesive on dentin bonding *Journal of Dentistry* **30(2-3)** 83-90.

14. Camps J & Pashley DH (2000) Buffering action of human dentin *in vitro Journal of Adhesive Dentistry* **2(1)** 39-50.

15. Erhardt MC, Osorio E, Aguilera FS, Proenca JP, Osorio R & Toledano M (2008) Influence of dentin acid-etching and NaOCl-treatment on bond strengths of self-etch adhesives *American Journal of Dentistry* **21(1)** 44-48.

16. Duarte S Jr, Perdigao J & Lopes MM (2006) Effect of dentin conditioning time on nanoleakage *Operative Dentistry* **31(4)** 500-511.

17. Velasquez LM, Sergent RS, Burgess JO & Mercante DE (2006) Effect of placement agitation and placement time on the shear bond strength of three self-etching adhesives *Operative Dentistry* **31(4)** 426-430.

18. Pashley DH, Ciucchi B, Sano H, Carvalho RM & Russell CM (1995) Bond strength versus dentin structure: A modeling approach *Archives of Oral Biology* **40(12)** 1109-1118.

19. Pashley DH & Carvalho RM (1997) Dentine permeability and dentine adhesion *Journal of Dentistry* **25(5)** 355-372.

20. Osorio R, Pisani-Proenca J, Erhardt MC, Osorio E, Aguilera FS, Tay FR & Toledano M (2008) Resistance of ten contemporary adhesives to resin-dentine bond degradation *Journal of Dentistry* **36(2)** 163-169.

21. 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(6)** 454-458.

22. Moszner N, Salz U & Zimmermann J (2005) Chemical aspects of self-etching enamel-dentin adhesives: A systematic review *Dental Materials* **21(10)** 895-910.

23. Toledano M, Proença JP, Erhardt MC, Osorio E, Aguilera FS, Osorio R & Tay FR (2007) Increases in dentin-bond strength if doubling application time of an acetone-containing one-step adhesive *Operative Dentistry* **32(2)** 133-137.

24. 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(2)** 126-134.

25. Sundfeld RH, Valentino TA, de Alexandre RS, Briso AL & Sundefeld ML (2005) Hybrid layer thickness and resin tag length of a self-etching adhesive bonded to sound dentin *Journal of Dentistry* **33(8)** 675-681.

26. el-Din AK & Abd el-Mohsen MM (2002) Effect of changing application times on adhesive systems bond strengths *American Journal of Dentistry* **15(5)** 321-324.

27. Koibuchi H, Yasuda N & Nakabayashi N (2001) Bonding to dentin with a self-etching primer: The effect of smear layers *Dental Materials* **17(2)** 122-126.

28. 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(3)** 215-235.

29. Miyazaki M, Onose H, Ida N & Kazama H (2003) Determination of residual double bonds in resin-dentin interface by Raman Spectroscopy *Dental Materials* **19(3)** 245-251.

30. Van Landuyt KL, De Munck J, Snauwaert J, Coutinho E, Poitevin A, Yoshida Y, Inoue S, Peumans M, Suzuki K, Lambrechts P & Van Meerbeek B (2005) Monomer solvent phase separation in one-step self-etch adhesives *Journal of Dental Research* **84(2)** 183-188.

31. Tay FR & Pashley DH (2001) Aggressiveness of contemporary self-etching adhesives. I: Depth of penetration beyond smear layers *Dental Materials* **17(4)** 296-308.

32. Hannig M, Reinhardt KJ & Bott B (1999) Self-etching primer vs phosphoric acid: An alternative concept for composite-to-enamel bonding *Operative Dentistry* **24(3)** 172-180.

33. Nakaoki Y, Sasakawa W, Horiuchi S, Nagano F, Ikeda T, Tanaka T, Inoue S, Uno S, Sano H & Sidhu SK (2005) Effect of double-application of all-in-one adhesives on dentin bonding *Journal of Dentistry* **33(9)** 765-772.

34. Rueggeberg FA & Margeson DH (1990) The effect of oxygen inhibition on an unfilled/filled composite system *Journal of Dental Research* **69(10)** 1652-1568.

35. Van Landuyt KL, Snauwaert J, De Munck J, Peumans M, Yoshida Y, Poitevin A, Coutinho E, Suzuki K, Lambrechts P & Van Meerbeek B (2007) Systematic review of the chemical composition of contemporary dental adhesives *Biomaterials* **28(26)** 3757-3785.

36. Can Say E, Nakajima M, Senawongse P, Soyman M, Ozer F, Ogata M & Tagami J (2006) Microtensile bond strength of a filled vs unfilled adhesive to dentin using self-etch and total-etch technique *Journal of Dentistry* **34(4)** 283-291.

37. Choi KK, Condon JR & Ferracane JL (2000) The effects of adhesive thickness on polymerization contraction stress of composites *Journal of Dental Research* **79(3)** 812-817.

38. Zheng L, Pereira PN, Nakajima M, Sano H & Tagami J (2001) Relationship between adhesive thickness and microtensile bond strength *Operative Dentistry* **26(1)** 97-104.

39. Yiu CK, Pashley EL, Hiraishi N, King NM, Goracci C, Ferrari M, Carvalho RM, Pashley DH & Tay FR (2005) Solvent and water retention in dental adhesive blends after evaporation *Biomaterials* **26(34)** 6863-6872.