Original Article

Effect of Endodontic Irrigants on Microtensile Bond Strength to Dentin After Thermocycling and Long-Term Water Storage

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Abstract

Objective: The bond strength of adhesives in irrigated dentin behaves differently over time. The aim of this study was to evaluate the influence of long-term water storage and thermocycling on the microtensile bond strength of adhesive systems to dentin irrigated with endodontic solutions.

Materials and Methods: Sixty human molars were used after removal of the occlusal portion and exposure of the dentin by grinding. The specimens were irrigated with 2.5% NaOCl for 30 minutes and then 17% EDTA for 5 minutes and assigned to six groups according to the adhesive system (n=10): G1 and G2–Clearfil SE Bond; G3 and G4–Single Bond 2; and G5 and G6–XP Bond. The teeth were restored with composite and were subjected to water storage for different time periods. G1, G3 and G5 were stored for 24 h; G2, G4 and G6 were stored for 6 months and were subjected to thermocycling (12,000 cycles, 5°C to 55°C, 500 cycles per week for 6 months). After storage, the tooth/restoration assembly was sectioned to obtain four sticks of approximately 1 mm^2, for microtensile bond strength testing. The results were analyzed by two-way ANOVA and Tukey's test.

Results: Significant differences were observed among the adhesives ($p<0.01$). No significant differences were observed in the microtensile bond strength between samples after 24 hours of storage without thermocycling and after 6-month storage with 12,000 cycles ($p<0.05$).

Conclusion: The bond strengths of G5 and G6 after irrigation with 2.5% NaOCl and 17% EDTA were significantly different from those of other groups. Long-term water storage/thermocycling had no effect on bond strength to dentin.

Key Words: Microtensile Bond Strength; Self-etch; Total-etch; NaOCl, EDTA

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INTRODUCTION

Root canal irrigation during endodontic treatment is necessary for successful chemical-mechanical preparation [1,2]. The function of endodontic irrigants is to facilitate preparation and disinfect the root canal [2].

These irrigants should not affect the dentin surface or interfere with restorative materials [3-6]. The deleterious effects of certain irrigators, such as sodium hypochlorite, have been confirmed by previous studies, which have shown that bond strength is decreased [3,5-7].

Sodium hypochlorite is an alkaline solution [8] with a wide antibacterial spectrum and sporicidal and virucidal potential [9].

The concentration ranges between 0.5 and 5.25%, and its use is conditioned by factors such as time and the type of instruments used [10].

Another substance used as an irrigant is EDTA, which is a chelating agent capable of decalcifying dentin after 5 minutes of use, at 20 to 30 micrometers of depth [8].

Regardless of the category of the adhesive system, current adhesion depends on the interaction between dental adhesives and the smear layer [11]. Etch-and-rinse adhesives remove the smear layer and superficial hydroxyapatite through etching with a separate acid gel. Self-etch adhesives render the smear layer permeable without removing it completely [11].

Adhesion durability is a parameter of the success of restorations. Degradation of the adhesive interface was demonstrated by Shono et al. [12], who found decreased bond strength as a function of storage time.

A commonly used in vitro aging technique is thermocycling that simulates the ingestion of hot and cold foods [13].

Given the importance of dentin sealing for a better prognosis with endodontic treatment, the evidence from previous studies that irrigant substances directly affect adhesion [2,3,4] and the lack of information about the effects of endodontic irrigants on dentin after adhesive interface degradation, the aim of this in vitro study was to evaluate the influence of long-term water storage and thermocycling on the microtensile bond strength of adhesive systems to dentin irrigated with endodontic solutions.

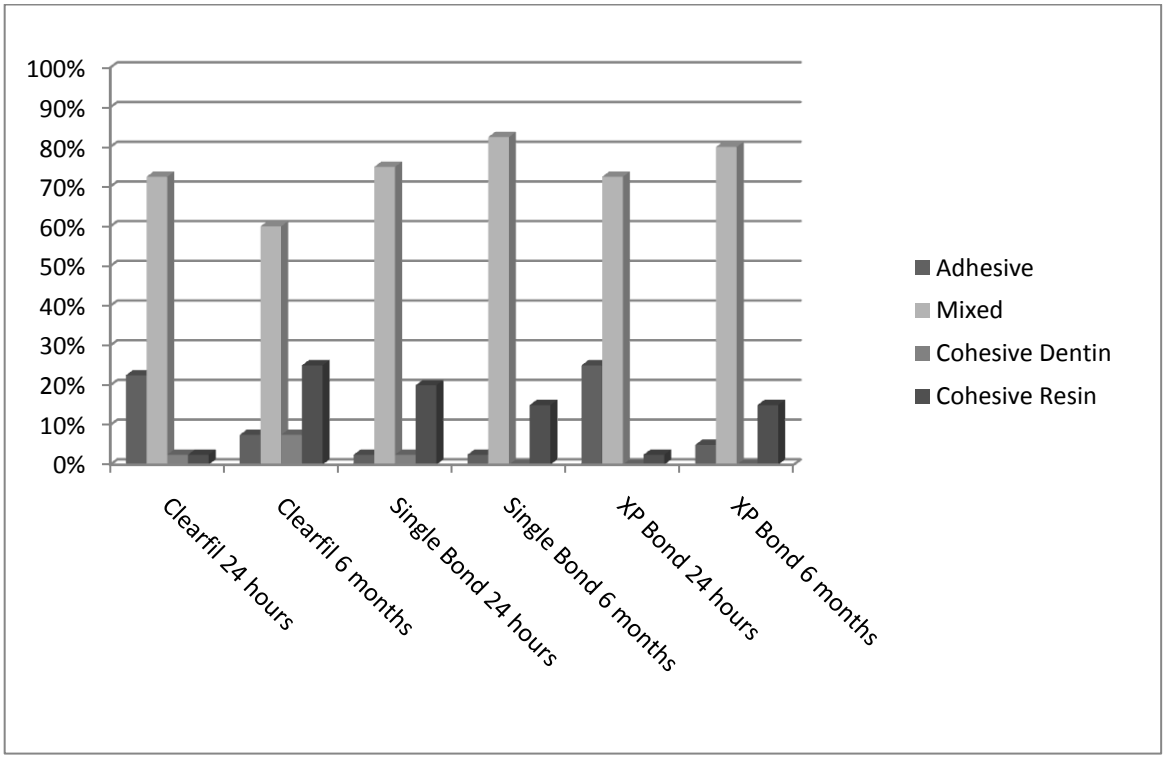
MATHERIALS AND METHODS

Sixty caries-free, extracted human third molars were selected for this study (obtained from the bank of teeth FORP-USP). The following protocol was prescribed, revised and approved by the Committee of Ethics in Human Research (2008.1.157.58.8). The teeth were stored in 0.2% thymol solution at 4°C for up to 6 months after extraction.

The teeth were embedded in polyester resin (Milflex ind. Química LTDA., São Bernardo do Campo, SP, Brazil) using plastic cylinders (15 mm in height and 20 mm in diameter), 1 mm apical to the limit between the crown and the root. Next, the teeth were mounted in a cutting machine (Isomet 1000, Buehler Ltd. Lake Bluff, IL, USA) and were sectioned to remove the occlusal surface at the middle third. The dentin surfaces were planed using #400 grit silicon carbide sandpaper (Buehler Ltd., Lake Bluff, IL, USA) under water cooling. The polishing procedure was performed until the deep dentin was exposed, as identified by observation of the pulp horn by transparency. To produce standardized smear layers, the teeth were subjected to polishing using #600 grit silicon carbide sandpaper (Buehler Ltd., Lake Bluff, IL, USA) under water cooling for 15 seconds.

The specimens previously obtained were divided randomly into six experimental groups. Subsequently, the teeth were immersed in 2.5% NaOCl (Farmácia da Terra, Ribeirão Preto, SP, Brazil) for 30 minutes [14] and then immersed in 17% EDTA (Merck, Darmstadt, Germany) [15] for 5 minutes and rinsed in deionized water for 10 seconds in order to simulate the amount of time that dentin is in contact with endodontic irrigants.

The adhesive systems were applied to the dentin surfaces following endodontic irrigation, according to the manufacturers' instructions (Table 1). The specimens in groups G1 and G2 were bonded with Clearfil SE Bond - CF (Kuraray Co. Ltd., Umeda, Osaka, Japan) that was light cured for 10 seconds.



Graph 1. Percent of fracture patterns for the groups

Table 1. Adhesive Systems, Composition, and Batch Number

Adhesive System	Chemical Composition	Batch Number	Adhesive Manipulation
XP Bond (Dentsply)	PENTA, UDMA, TEGDMA, HEMA, TCB resin, t-butanol, Functional amorphous silica, CQ, Butylated benzenediol (stabilizer), Ethyl-4-dimethylaminobenzoate	0707002474	Two consecutive coats of the adhesive system were applied using a disposable applicator with gentle agitation for 20 seconds, followed by a 5-second gentle air stream and light curing
Single Bond 2 (3M ESPE)	Dimethacrylates, HEMA, polyalkenoid acid copolymer, 5 nm silane treated colloidal silica, ethanol, water, photo-initiator	7MY	Two consecutive coats of the adhesive system were applied using a disposable applicator with gentle agitation for 20 seconds, followed by a 5-second gentle air stream and light curing
Clearfil SE Bond (Kuraray)	PRIMER MDP, HEMA, hydrophilic dimethacrylate, photo-initiator, water BOND MDP, Bis-GMA, HEMA, Hydrophobic aliphatic dimethacrylate, photo-initiators, silanated colloidal silica	00727° 01044A	The primer was applied for 20 seconds, followed by the application of the adhesive, gentle air-drying for 5 seconds, and light curing for 10 seconds

PENTA – Phosphoric acid modified acrylate resin; UDMA – Urethane Dimethacrylate; TEGDMA – Triethyleneglycol dimethacrylate; HEMA – 2-hydroxyethylmethacrylate; TCB resin –Carboxylic acid modified dimethacrylate; CQ – Camphorquinone; MDP – 10-methacryloyloxydecyl dihydrogen phosphate, Bis-GMA – bisphenol-A diglycidyl methacrylate

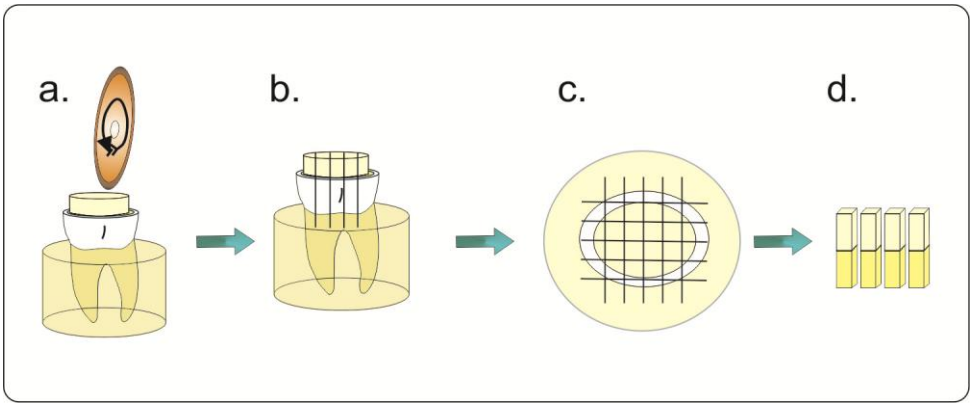


Fig 1. Schematic of sticks preparation (a) and (b) mesio-distal cut, (c) bucal-lingual cut, (d) sticks.

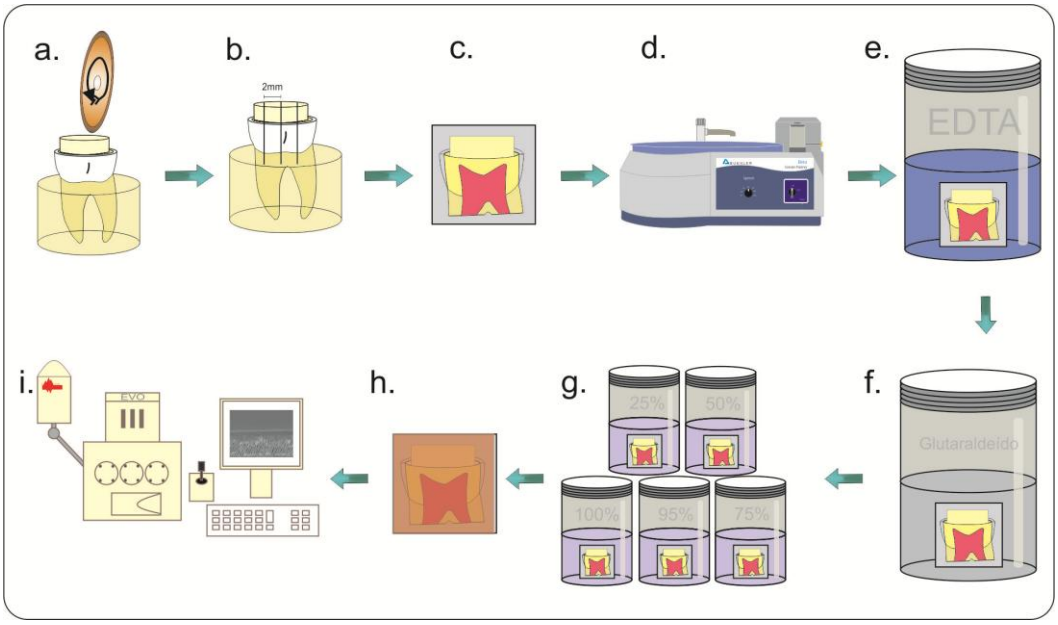


Fig2. Schematic of SEM preparation. (a) and (b): 2 mm cut; (c) embedded in a polyester resin; (d) surface polishing; (e) EDTA cleaning; (f) fixation with glutaraldehyde; (g) alcohol dehydration; (h) gold sputter-coated; (i) SEM analysis

The specimens in groups G3, G4, G5 and G6 were conditioned with 37% phosphoric acid (Dentsply, York, PA, USA) for 15 seconds and rinsed for 30 seconds. Then, the excess water was removed with absorbent paper, keeping the dentin moist. In groups G3 and G4, Single Bond 2 - SB (3M Dental Products, St. Paul, MN, USA) was applied, and in G5 and G6, XP Bond -XP (Dentsply, York, PA, USA) was applied and light cured (Demetron/Kerr, Danbury, USA) for 10 seconds.

The teeth were restored using a microhybrid composite resin (Z100, 3M ESPE, shade A3, St. Paul, MN, USA).

Composite resin blocks (4 mm in height) were built with 4 increments of 1 mm thickness each. Each increment was light cured for 20 seconds, in a continuous mode as recommended by the manufacturer.

Then, the specimens were kept in deionized water at 37oC for the respective storage times. After 24 hours, the teeth in groups G1, G2 and

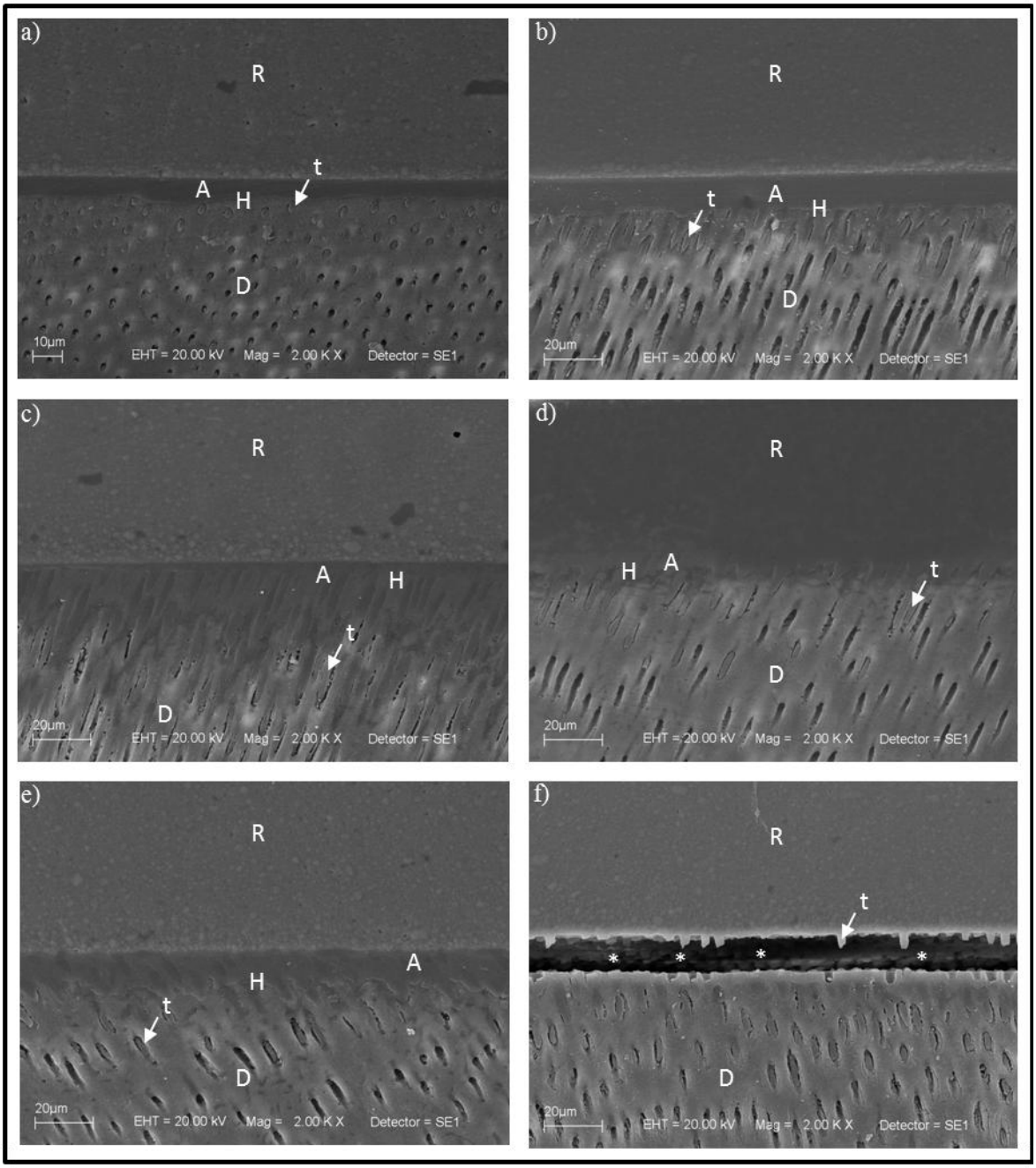


Fig3. Representative SEM micrographs of the adhesive interface (2000X magnification) (a) Clearfil SE Bond – 24 hours; (b) Clearfil SE Bond after 6 months storage/12,000 thermocycles; (c) Single Bond 2 – 24 hours; (d) Single Bond 2 after 6 months storage/12,000 thermocycles, (e) XP Bond – 24 hours, and (f) XP Bond after 6 months storage/12,000 thermocycles. R = Composite Resin, D = Dentin, t = tag, H = Hybrid Layer, * = Interface gap

G3 were attached to a cutting machine and were sectioned using a low-speed diamond saw; then, they were rotated 90o to produce sticks (1.0±0.2 mm2).

Approximately 8 sticks were obtained per tooth. No premature failures were observed during sectioning. Four sticks were selected from the central portion of each tooth. Therefore, 40 sticks were obtained for each group (Fig 1). For statistical purposes, the mean bond strength of all four sticks was considered.

The specimens from groups G2, G4 and G6 were subjected to thermocycling (12,000 cycles, 5°C to 55°C, 30 second dwell time, 500 cycles per week for 6 months) (Ética Equipamentos Científicos S.A., São Paulo, SP, Brazil). The specimens were kept in deionized water at 37oC between the cycles that was replaced weekly. At the end of water storage and thermocycling, the samples were cut into sticks according to the aforementioned technique.The sticks were attached to a Universal Testing Machine (EMIC Equipamentos e Sistemas de Ensaio LTDA, São José dos Pinhais, PR, Brazil), using a cyanoacrylate-based adhesive (Super Bonder, Henkel Ltda., São Paulo, SP, Brazil). The test was performed at a speed of 0.5 mm/min, using a 500 N load cell.

The bond strengths were recorded in MPa, and the cross-section areas of the sticks were measured using digital calipers (Mitutoyo, Tokyo, Japan).

Statistical analysis was performed using two-way ANOVA and Tukey's test at a 5% significance level.

Fracture types were determined using a stereomicroscope (40x magnification) (Leica S6 D Stereozoom, Leica Microsystems AG, Heerbrugg, Switzerland).

Failures were classified as adhesive (fracture of the dentin-adhesive interface), cohesive in dentin (fracture of dentin structure), cohesive in resin (resin fracture), and mixed (combination of the previous types).

One tooth from each group was used. The specimens were then sectioned into two slabs of ~2 mm. The slabs were embedded in polyester resin, sanded with ascending grains (#600, #1200, #2400) and polished with 3 µm alumina paste (Struers A/S, Copenhagen, Denmark).

The specimens were placed in an ultrasonic cleaner (1440D, Odontobras, Ribeirão Preto, Brazil) and cleaned with EDTA for 5 minutes, fixed in glutaraldehyde for 24 h, dehydrated in a battery of increasing concentrations of alcohol, and gold sputter coated (Fig 2).

RESULTS

Two-way ANOVA revealed statistically significant differences among adhesives (p=0.007). There were no statistically significant differences with regard to aging (p=0,088) or the interaction (p=0.226) between the adhesives and aging (Table 2).

Table 2. Mean Microtensile Bond Strength Values (MPa) and Standard Deviations

	24 Hours/ No Cycling	**6 Months/12.000 Thermocycling**
Clearfil SE Bond	34.34 (12.46)^{Aab}	42.76 (15.43)^{Aa}
Single Bond	43.20 (15.00)^{Aa}	41.17 (12.95)^{Aa}
XP Bond	27.09 (15.67)^{Ab}	35.31 (13.86)^{Aa}

Capital letters indicate analysis by lines, and lower case indicates analysis by columns. The same letters indicate statistically similar values.

XP presented lower microtensile bond strength compared to SB ($p<0.01$), and it was similar to CF at 24 hours. At 6 months, the adhesive systems were similar (Table 2).

There were no statistically significant differences between the microtensile bond strengths of the specimens stored for 24 hours and those of the same group analyzed after 6 months of water storage and 12,000 thermal cycles (Table 2).

The analysis of the fracture patterns revealed that the predominant type of fracture was mixed: 72.5% in G1; 60% in G2; 75% in G3; 82.5% in G4; 72.5% in G5; and 80% in G6.

DISCUSSION

Endodontic irrigant solutions are applied to dissolve organic tissue, act as antimicrobials and help to debride the canal system because they are non-toxic to periapical tissues [16]. However, some studies have shown that these substances can influence the quality of adhesion to dentin [6,7], particularly when using endodontic solutions at higher concentrations [10].

Coronal sealing is essential for successful endodontic therapy [17,18]. Adhesive materials have also contributed to improving coronal sealing and increasing the retention of restorative materials [19] to dentin walls, increasing the possibility of marginal sealing, mechanical resistance to mastication stress and the durability of restorations [19,20]. The adhesive systems used in this study were selected because of their proven successful results, obtained in both in vitro [21,22] and in vivo studies [21,23] Another factor for the use of these adhesives was their different solvent contents, as shown in Table 2.

Time can have a negative effect on the durability of the coronal sealing of composite resin restorations [13,24]. Thermocycling accelerates the hydrolysis of the adhesive interface components and enhances the coefficient of thermal expansion of the restorative material, producing stress at the interface [25-28].

It has been estimated that 10,000 cycles correspond to 1 year of in vivo activity [17].

However, our results showed that 6 months and 12,000 thermal cycles did not decrease the bond strength of dentin irrigated with 2.5% NaOCl and EDTA. Thus, the application of EDTA, which provided smear layer removal [10] and create demineralized dentin zones 2-4 [29], might have caused these results, thus allowing greater interaction of the adhesive systems with dentin and enabling adhesive maintenance independent of the adhesive system.

According to Myiasaki et al. [30], a combination of thermocycling and storage in deionized water can simulate the tension generated in the oral cavity. In addition, it has been shown that water storage significantly reduces bond strength values after short periods of time [31]. SEM micrographs (Fig 3) revealed significant interaction of the adhesive systems with demineralized dentin. The SB and CF adhesive systems presented similar hybrid layers and evident resin tags, even after thermocycling; however, the presence of an interfacial gap was observed with the XP adhesive system. The gap observed under SEM might have been the result of the polishing of the samples, and the vacuuming could also have damaged the bond interface, resulting in its rupture [32].

Cohesive failures were found in only 15.41% of the samples. This low incidence of cohesive failures corroborates the findings of another study [23]. The high number of mixed fractures (Graphic 1) could have resulted from strong interaction between the adhesives and the dentin substrate, as evidenced in the SEM analysis (Fig 3) that showed hybrid layer formation and the presence of tags for the adhesive systems.

Decrease bond strengths have been shown elsewhere after irrigation of dentin with 5.25% sodium hypochlorite for 30 minutes [6]. Other in vitro studies have found that the bond strengths were reduced after the application of 5% sodium hypochlorite for 1 minute [4,33].

This finding indicates that as long as there is contact with the dentin, irrigant solution reduces adhesive strength.

Studies have shown that when 5% NaOCl has been applied to dentin, self-etch adhesive systems have better bond strength values, compared to etch-and-rinse adhesives [4,6,17,33]. These findings differ from those of the present study that applied to 2.5% NaOCl and 17% EDTA and displayed the best values in teeth restored with Single Bond, suggesting that the adhesive system could be dependent on the irrigating solution's concentration.

XP exhibited the lowest bond strength values, with a significant difference from the SB adhesive system; however, both systems were similar to CF.

A possible explanation for this finding is the lower amount of calcium present in dentin after the application of 2.5% hypochlorite and EDTA. According to Sayin et al. [34], the application of combined solutions promotes greater removal of calcium from dentin. The presence of calcium plays an important role in the binding of adhesives [34].

If an adhesive system has the monomer PENTA that has strong affinity for calcium in its composition, a lower quantity of calcium could decrease the bond strength of this adhesive system [35,36].

This behavior was also observed for the 10-MDP monomer present in Clearfil SE Bond that showed a statistical similarity to XP Bond at 24 hours. A possible explanation for the higher bond strength of Single Bond is the carboxylic group of polyalkenoic acid present in this adhesive system, as well as hydroxyapatite on the dentin surface or incompletely dissolved hydroxyapatite on exposed collagen fibrils forming ionic bonds that could have contributed to resin-dentin bonds [37, 38]. Further studies, using mechanical degradation of the adhesive interface over time, are required to evaluate the bond strength of dentin irrigated with endodontic substances.

CONCLUSION

Under the limitations of this in vitro study, it can be concluded that the bond strength of dentin irrigated with 2.5% NaOCl and 17% EDTA is influenced by the adhesive system used. Aging by thermocycling and water storage did not interfere with the bond strength of the tested adhesive systems.

REFERENCES

1- Marending M, Luder HU, Brunner TJ, Knecht S, Stark WJ, Zehnder M. Effect of sodium hypochlorite on human root dentine - mechanical, chemical and structural evaluation. Int Endod J. 2007 Oct;40(10):786-93.

2- Zehnder M. Root canal irrigants. J Endod. 2006 May;32(5):389-98.

3- Fawzi EM, Elkassas DW, Ghoneim AG. Bonding strategies to pulp chamber dentin treated with different endodontic irrigants: microshear bond strength testing and SEM analysis. J Adhes Dent. 2010 Feb;12(1):63-70.

4- Ozturk B, Özer F. Effect of NaOCl on bond strengths of bonding agents to pulp chamber lateral walls. J Endod. 2004 May;30(5):362-5.

5- Pelegrine RA, De Martin AS, Cunha RS, Pelegrine AA, da Silveira Bueno CE. Influence of chemical irrigants on the tensile bond strength of an adhesive system used to cement glass fiber posts to root dentin. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010 Nov;110(5):e73-6.

6- Santos JN, Carrilho MR, De Goes MF, Zaia AA, Gomes BP, Souza-Filho FJ et al. Effect of chemical irrigants on the bond strength of a self-etching adhesive to pulp chamber dentin. J Endod. 2006 Nov;32(11):1088-90.

7- Nikaido T, Takano Y, Sasafuchi Y, Burrow MF, Tagami J. Bond strengths to endodontically-treated teeth. Am J Dent. 1999 Aug;12(4):177-80.

8- Silva LA, Leonardo MR, Assed S, Tanomaru Filho M. Histological study of the effect of some irrigating solutions on bacterial endotoxin in dogs. Braz Dental J. 2004;15(2):109-14.

9- McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev. 1999 Jan;12(1):147-79.
10- Spanó JC, Barbin EL, Santos TC, Guimarães LF, Pécora JD. Solvent action of sodium hypochlorite on bovine pulp and physico-chemical properties of resulting liquid. Braz Dent J. 2001;12(3):154-7.
11- Perdigão J. New developments in dental adhesion. Dent Clin North Am. 2007 Apr;51(2):333-57.
12- Shono Y, Terashita M, Shimada J, Kozono Y, Carvalho RM, Russell CM et al. Durability of resin-dentin bonds. J Adhes Dent. 1999 Autumn;1(3):211-8.
13- Gale MS, Darvell BW. Thermal cycling procedures for laboratory testing of dental restorations. J Dent. 1999 Feb;27(2):89-99.
14- Tirali RE, Turan Y, Akal N, Karahan ZC. In vitro antimicrobial activity of several concentrations of NaOCl and Octanisept in elimination of endodontic pathogens. Oral Sur Oral Med Oral Pathol Oral Radiol Endod. 2009 Nov;108(5):e117-20.
15- Cunha RS, De Martin AS, Barros PP, da Silva FM, Jacinto Rde C, Bueno CE. In vitro evaluation of the cleansing working time and analysis of the amount of gutta-percha or Resilon remnants in the root canal walls after instrumentation for endodontic retreatment. J Endod. 2007 Dec;33(12):1426-8.
16- Cheung GS, Stock CJ. In vitro cleaning ability of root canal irrigants with and without endosonics. Int Endod J. 1993 Nov;26(6):334-43.
17- Belli S, Zhang Y, Pereira PN, Pashley DH. Adhesive sealing of the pulp chamber. J Endod. 2001 Aug;27(8):521-6.
18- Ozturk B, Özer F, Belli S. An in vitro comparison of adhesive systems to seal pulp chamber walls. Int Endod J. 2004 May;37(5):297-306.
19- Cecchin D, Farina AP, Galafassi D, Barbizam JV, Corona SA, Carlini-Júnior B. Influence of sodium hypochlorite and edta on the microtensile bond strength of a self-etching adhesive system. J Appl Oral Sci. 2010 Jul-Aug;18(4):385-9.
20- Fortin D, Swift EJ Jr, Denehy GE, Reinhardt JW. Bond strength and microleakage of current dentin adhesives. Dent Mater. 1994 Jul;10(4):253-8.
21- Hebling J, Castro FL, Costa CA. Adhesive performance of dentin bonding agents applied in vivo and in vitro. Effect of intrapulpal pressure and dentin depth. J Biomed Mater Res B Appl Biomater. 2007 Nov;83(2):295-303.
22- Manhart J, Trumm C. Marginal adaptation of an etch-and-rinse adhesive with a new type of solvent in class II cavities after artificial aging. Clin Oral Investig. 2010 Dec;14(6):699-705.
23- Frankenberger R, Krämer N, Lohbauer U, Nikolaenko SA, Reich SM. Marginal integrity: is the clinical performance of bonded restorations predictable in vitro?. J Adhes Dent. 2007;9 Suppl 1:107-16.
24- Giannini M, Seixas CA, Reis AF, Pimenta LA. Six-month storage-time evaluation of one-bottle adhesive systems to dentin. J Esthet Restor Dent. 2003;15(1):43-8.
25- Bedran-de-Castro AK, Pereira PN, Pimenta LA, Thompson JY. Effect of thermal and mechanical load cycling on microtensile bond strength of a total-etch adhesive system. Oper Dent. 2004 Mar-Apr;29(2):150-6.
26- De Munck J, Van Landuyt K, Peumans M, Poitevin A, Lambrechts P, Braem M et al. A critical review of the durability of adhesion to tooth tissue: methods and results. J Dent Res. 2005 Feb;84(2):118-32.
27- Dos Santos PA, Garcia PP, Palma-Dibb RG. Shear bond strength of adhesive systems to enamel and dentin. Thermocycling influence. J Mater Sci Mater Med. 2005 Aug;16(8):727-32.
28- Ebert J, Löffler C, Roggendorf MJ, Petschelt A, Frankenberger R. Clinical adhesive sealing of the pulp chamber following endodontic treatment: influence of thermomechanical loading on microleakage. J Adhes Dent. 2009 Aug;11(4):311-7.

29- García-Godoy F, Loushine RJ, Itthagarun A, Weller RN, Murray PE, Feilzer AJ et al. Application of biologically-oriented dentin bonding principles to the use of endodontic irrigants. Am J Dent. 2005 Aug;18(4):281-90.
30- Miyazaki M, Sato M, Onose H, Moore BK. Influence of thermal cycling on dentin bond strength of two-step bonding systems. Am J Dent. 1998 Jun;11(3):118-22.
31- Okuda M, Pereira PN, Nakajima M, Tagami J, Pashley DH. Long-term durability of resin dentin interface: nanoleakage vs. microtensile bond strength. Oper Dent. 2002 May-Jun;27(3):289-96.
32- Van Meerbeek B, Vargas M, Inoue S, Yoshida Y, Perdigão J, Lambrechts P et al. Microscopy investigations. Techniques, results, limitations. Am J Dent. 2000 Nov:13 (Spec No):3D-18D.
33- Erdemir A, Ari H, Güngüneş H, Belli S. Effect of medications for root canal treatment on bonding to root canal dentin. J Endod. 2004 Feb;30(2):113-6.
34- Sayin TC, Serper A, Cehreli ZC, Kalayci S. Calcium loss from root canal dentin following EDTA, EGTA, EDTAC, and tetracycline-HCl treatment with or without subsequent NaOCl irrigation. J Endod. 2007 May;33(5):581-4.
35- Inai N, Kanemura N, Tagami J, Watanabe LG, Marshall SJ, Marshall GW. Adhesion between collagen depleted dentin and dentin adhesives. Am J Dent. 1998 Jun;11(3):123-7.
36- Toledano M, Perdigão J, Osorio R, Osorio E. Effect of dentin deproteinization on microleakage of Class V composite restorations. Oper Dent. 2000 Nov-Dec;25(6):497-504.
37- Kim DS, Park SH, Choi GW, Choi KK, Kim SY. Effect of EDTA treatment on the hybrid layer durability in total-etch dentin adhesives. Dent Mater J. 2011;30(5):717-22.
38- Fukuda R, Yoshida Y, Nakayama Y, Okazaki M, Inoue S, Sano H et al. Bonding efficacy of polyalkenoic acids to hydroxyapatite, enamel and dentin. Biomaterials. 2003 May;24(11):1861-7.