Evaluation of Topical Pre-Fluoride Therapy Effects on Marginal Microleakage of Composite Restorations in Deciduous Teeth: An In Vitro Study

Mina Biria1, Hasan Torabzadeh2, Elmira Ghazanfari3, Atefeh Gohari4, Negin Eslami Amirabadi5, Sanaz Kamareh6*

1 Associate Professor, Department of Pediatric Dentistry, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2 Preventive Dentistry Research Center, Research Institute of Dental Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3 Dentist, Private Practice, Tehran, Iran
4 Postgraduate Student, Department of Endodontics, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran
5 Dentistry Student, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran
6 Postgraduate Student, Department of Pediatric Dentistry, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Abstract

Background and Aim: Since fluoride therapy is usually the first step of treatment in pediatric dentistry and it may interfere with other treatments, such as composite filling, this study aimed to evaluate the possible effects of topical pre-fluoride therapy on marginal microleakage of composite restorations in deciduous teeth.

Materials and Methods: In this in-vitro study, 30 deciduous canines were randomly allocated to five groups: 1.23% acidulated phosphate fluoride (APF) gel was used in two groups, while 2% sodium fluoride (NaF) gel was used in the other two groups. Thirty minutes and two weeks after fluoride therapy, Class V cavities were prepared and restored using composite resins. After thermocycling, the teeth were soaked in 0.5% fuchsin solution and were sagittally sectioned in half. The extension of dye penetration into the occlusal and gingival walls was investigated under a stereomicroscope at ×30 magnification and scored using a 0-3 scoring system. Data were analyzed using Kruskal-Wallis and Wilcoxon signed-rank tests.

Results: Marginal microleakage was neither affected in enamel walls (P=0.213) nor in dentinal walls (P=0.851). The scores of microleakage in enamel walls were lower than that in dentinal walls, and this difference was statistically significant (P<0.05).

Conclusion: Topical fluoride therapy using 1.23% APF or 2% NaF gel before the placement of composite resin restorations has no negative effect on marginal microleakage.

Key Words: Fluoride, Deciduous Teeth, Composite Resin, Dental Leakage

Introduction

Currently, the adhesion of bonding agents has been improved significantly, which makes tooth-colored restorative materials a popular choice for aesthetic restorations [1]. All resin-based restorative materials have some...
degrees of polymerization shrinkage, which result in contraction stress at the interface between the restoration and the cavity walls. The consequent marginal gap provides a space for penetration of bacteria, fluids, molecules, and ions, which causes marginal discoloration, tooth hypersensitivity, pulpal inflammation, recurrent caries, and consequently, failure of the restoration [2].

The term “microleakage” refers to the penetration of bacteria, molecules, and ions into the gap between the restoration and the cavity walls. Marginal microleakage is still one of the most paramount complications of composite resin restorations [2]. Fluoride-containing products play an important role in caries prevention and remineralization improvement. In pediatric dentistry, usually, fluoride therapy is the first step of treatment, which is carried out prior to any other therapeutic procedure [3]. At the first appointment, it is necessary to familiarize children with dental instruments and procedures; this can be performed by a simple and non-invasive fluoride therapy, which can help to reduce patient stress and provide better cooperation [3]. 5% sodium fluoride (NaF) varnishes, 2% NaF gel, and 1.23% acidulated phosphate fluoride (APF) gel are the most common compounds used in professional fluoride therapy [4].

In some situations, composite resin restorations should be placed immediately after fluoride therapy, and in many cases, a few days after fluoride therapy [3]. Consequently, possible negative effects of fluoride compounds on the physical properties of tooth-colored restorations remain as a concern. The adverse effects of acidic or neutral compositions of fluoride gels should also be investigated.

Some clinical and in-vitro studies have shown that topical fluoride therapy does not interfere with the bonding of fissure sealants to enamel [5]. Shabzendedar et al [6] (2011) showed that post-restorative fluoride therapy with APF gel increases the microleakage of glass ionomer restorations in permanent teeth. Tabari et al [7] (2011) indicated that fluoride therapy with APF gel after restoration placement does not have any significant effects on the microleakage of Tetric Flow® composite resins and Helioseal® fissure sealants in permanent teeth. They suggested that APF gel can be used routinely in dental treatments [7].

Moosavi et al [3] (2010) also investigated the effects of pre- and post-treatment with APF gel on the microleakage of aesthetic restorations (including composite resins and glass ionomers) in permanent teeth. They reported that pre- and post-topical fluoride therapy had no adverse effects on the marginal microleakage of composite resins. However, microleakage in enamel margins had increased significantly in glass ionomer restorations. Scanning electron microscopic (SEM) investigations showed that marginal integrity was significantly better on enamel margins than on dentinal ones [3]. Because of the difficulties in isolation, limitations in accessibility, and higher organic content of cementum, appropriate replacement of subgingival carious lesions with a restoration is a major concern in dentistry [3]. In many cases of Class V cavities of the primary teeth, the gingival floor extends under the gingiva. Therefore, evaluation of marginal microleakage in the subgingival floor of composite restorations in deciduous teeth is imperative.

This study was conducted to evaluate the possible effects of pre-treatment with the commonly used fluoride gels on the marginal microleakage of composite resin restorations in deciduous teeth in order to reveal effective fluoride compounds and the appropriate time interval between fluoride therapy and placement of tooth-colored restorative materials.

Materials and Methods
In this in-vitro study, 30 deciduous canines, extracted at least three months ago due to orthodontic treatment, were included with at least two-thirds of their root length remaining unresorbed. The sample size was calculated to be 12 in each of the five groups according to a previous study by Moosavi et al [3] and by using PASS II software (NCSS LLC, Kaysville, USA), considering alpha=0.05, beta=0.2, and effect size=0.5. 

All teeth were immersed in a 0.9% saline solution,
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which was changed daily. Debris, stain, calculi, and attached periodontal ligament (PDL) were removed from teeth surfaces, and samples were kept in 0.5% Chloramine-T solution at 4°C for 24 hours for disinfection. All included teeth were examined under a stereomicroscope (Carl Zeiss, Oberkochen, Germany) at ×10 magnification to check for caries, fillings, cracks, fractures, attrition, developmental anomalies such as enamel hypoplasia, and fluorosis. The selected teeth were randomly divided into five groups:

Group 1: Control group with no fluoride therapy.

Group 2: Topical fluoride treatment with 1.23% APF gel (Topex, Sultan Healthcare Inc., Englewood, NJ, USA) for 4 minutes and application of a composite restoration after 30 minutes.

Group 3: Topical fluoride treatment with 2% NaF (Neutral Fluoride Preventive Treatment Gel, Pascal International Inc., Bellevue, WA, USA) for 4 minutes and placement of a composite restoration after 30 minutes.

Group 4: Topical fluoride treatment with 1.23% APF gel for 4 minutes and application of a composite restoration after two weeks.

Group 5: Topical fluoride treatment with 2% NaF for 4 minutes and placement of a composite restoration after two weeks.

In each of the study groups, after the removal of excess fluoride gel using a cotton pellet, the samples were placed and kept in artificial saliva [NaCl (2.9 g), CaCl₂ (0.12 g), NaH₂PO₄ (0.13 g), NaF (5 cc, 100 parts-per-million (ppm)), Na₃[PO₄ (5 cc, 0.02%)]] until the cavity preparation time. In groups 2 and 3, 30 minutes after fluoride therapy, and in groups 4 and 5, two weeks after the application of fluoride gels, Class V cavities with 3×3×1-mm³ dimensions (mesiodistal width=3 mm, occlusogingival height=3 mm, and depth=1 mm) were prepared on the buccal and lingual surfaces of each tooth in groups 2, 3, 4, and 5 using a cylindrical diamond bur (F835-008C, ökoDENT, Germany) with a high-speed handpiece under copious water spray. Consequently, 12 cavities were prepared in each study group. The incisal and gingival walls of the cavities were located on the enamel and 0.5 mm below the cementoenamel junction (CEJ), respectively. Each diamond bur was used for five preparations. In order to standardize cavity sizes, the dimensions of each preparation were measured with a periodontal probe. Subsequently, 37% phosphoric acid gel (DiaEtch, DiaDent Europe, Almere, The Netherlands) was applied for 15-20 seconds on dentinal walls and for 30 seconds on enamel walls and rinsed with water for 15 seconds. Excess moisture was removed by gentle air-drying without over drying the dentinal surfaces. Two consecutive layers of dentin bonding agent (Adper Single Bond 2, 3M ESPE, St. Paul, MN, USA) were applied on the cavity walls. Then, the air was gently blown to uniformly diffuse each layer of dentin bonding agent on the tooth surface. Light-curing was performed for 20 seconds using a light-emitting diode (LED) light-curing unit (SDS Kerr Demetron, Danbury, CT, USA) with a light intensity of 760 mW/cm². Subsequently, Filtek Z250 composite (3M ESPE, St. Paul, MN, USA) was incrementally applied and cured for 40 seconds. The restoration surface was then polished using polishing burs (862-012, ökoDENT, Germany), and medium and fine finishing discs (No.1.622 and No.1.624, Stem Discs, Moscow, Russia). Each polishing bur or disc was used only for 10 teeth. All samples were then stored in a 0.9% saline solution in an incubator (PECO, Pooya Electronic Co., Tehran, Iran) at 37°C for 24 hours. The teeth were then subjected to 1000 thermal cycles at 5-55°C with a dwell time of 30 seconds and a transfer time of 10 seconds. Dye penetration technique was used to evaluate marginal microleakage. All samples were dried at room temperature. The apical 2 mm of the teeth’s roots was covered by melted glue wax, and then, all surfaces, except for the restoration surfaces and 1mm around the area, were covered with two layers of nail polish. The teeth were then soaked in 0.5% fuchsin solution at 37°C for 24 hours (Figure 1A), rinsed with water, and sagittally sectioned by a diamond disc (Mecatome T201A; Presi, Paris, France) in a buccolingual direction in the center of the restorations under water coolant (Figure 1B) [3].
Figure 1. (A) Teeth removed from 0.5% fuchsine solution after 24 hours. (B) Samples were sagittally cut in half.

Figure 2. (A) A view of a sample with no microleakage (grade 0). (B) A view of a sample with dye penetration into less than half of cavity depth (grade 1). (C) A view of a sample with dye penetration into more than half of cavity depth (grade 2). (D) A view of a sample with dye penetration into the tooth-restoration interface (grade 3).

The gingival and incisal margins of the restorations in each tooth were evaluated for microleakage under a stereomicroscope (Carl Zeiss, Oberkochen, Germany) at ×30 magnification. If any discrepancy in dye penetration was present between the two halves of a single tooth, the higher value would be recorded. The scoring system for dye penetration was as follows: 0 = No dye penetration, 1 = Dye penetration to the extent of half the cavity depth, 2 = Dye penetration extending to more than half of the cavity depth, and 3 = Dye penetration extending to the axial wall [7].

Kruskal-Wallis and Wilcoxon signed-rank tests were used for comparison of the calculated measures of microleakage between the groups. Data analysis was performed using SPSS 21 software (IBM Corp., Armonk, NY, USA).

Results

In this in-vitro study, the marginal microleakage of a total number of 24 margins was evaluated in each of the five groups. These margins included the gingival (dentinal) and occlusal (enamel) marginal walls of Class V restorations on both buccal and lingual surfaces of six deciduous canine teeth.

The scores of microleakage of each margin are presented in Table 1. Kruskal-Wallis test revealed no significant differences in the marginal microleakage of enamel walls between the groups (P = 0.213). Moreover, no statistically significant difference was present in the marginal microleakage of dentinal walls between the groups (P = 0.851). Wilcoxon signed-rank test showed significant differences in microleakage between occlusal and gingival walls in groups 1, 2, 4, and 5 (P < 0.05). However, the difference between occlusal and gingival walls was not significant in group 3 (P = 0.054; Table 1).

The generalized estimating equation (GEE) logistic regression model was used to evaluate the effects of different parameters, including the chemical composition of fluoride (1.23% APF versus 2% NaF), the evaluated cavity wall (occlusal versus gingival), and the interval between fluoride treatment and restoration placement (30 minutes versus 2 weeks). The results of the logistic regression showed that only the cavity wall significantly affected microleakage. The chance of microleakage in dentinal walls was 11.61 times more than that in enamel walls.

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**Table 1.** Frequency distribution and proportional frequency of microleakage of the groups in occlusal and gingival walls

| Grade of Micro leakage | Group 1 (Control) N(%) | Group 2 (1.23% APF; 30 minutes) N(%) | Group 3 (2% NaF; 30 minutes) N(%) | Group 4 (1.23% APF; 2 weeks) N(%) | Group 5 (2% NaF; 2 weeks) N(%) | P-value |
|------------------------|------------------------|--------------------------------------|----------------------------------|----------------------------------|--------------------------------|---------|
| Occlusal Microleakage  |                        |                                      |                                  |                                  |                                |         |
| 0                      | 12 (100)               | 9 (75)                               | 8 (66.7)                         | 9 (75)                           | 11 (91.7)                      | 0.213   |
| I                      | 0 (0)                  | 2 (16.7)                             | 4 (33.3)                         | 2 (16.7)                         | 1 (8.3)                        |         |
| II                     | 0 (0)                  | 1 (8.3)                              | 0 (0)                            | 1 (8.3)                          | 0 (0)                          |         |
| III                    | 0 (0)                  | 0 (0)                                | 0 (0)                            | 0 (0)                            | 0 (0)                          |         |
| 0                      | 5 (41.7)               | 1 (8.3)                              | 3 (25)                           | 3 (25)                           | 3 (25)                         | 0.851   |
| Gingival Microleakage  |                        |                                      |                                  |                                  |                                |         |
| I                      | 4 (33.3)               | 9 (75)                               | 7 (58.3)                         | 6 (50)                           | 8 (66.7)                       |         |
| II                     | 3 (25)                 | 2 (16.6)                             | 1 (8.3)                          | 3 (25)                           | 1 (8.3)                        |         |
| III                    | 0 (0)                  | 0 (0)                                | 1 (8.3)                          | 0 (0)                            | 0 (0)                          |         |

NaF=sodium fluoride, APF=acidulated phosphate fluoride

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Discussion

There is a growing tendency towards preventive dentistry and aesthetic restorative materials. Therefore, increased concern has been raised on the interference of fluoride therapy with composite resin bonding [8]. Marginal microleakage of composite resin restorations still remains a challenge, especially in margins formed in dentin or cementum [9].

The effect of fluoride therapy with APF before and after tooth restoration using tooth-colored materials on marginal microleakage in permanent teeth has been investigated in several studies. However, most of these studies investigated the effect of fluoride therapy after restoration. Due to structural differences between deciduous and permanent teeth, the results of these studies cannot be generalized to deciduous teeth. Furthermore, evaluating the effects of fluoride therapy using APF and NaF gels before the placement of tooth-colored restorations, especially with different intervals between fluoride therapy and restoration placement, is clinically imperative.

Resin shrinkage and voids have been reported in the resin matrix because of high concentrations of H and F ions released from APF [10]. In addition, the destructive effects of fluoride on the filler-matrix interface have been confirmed [11]. Fluoride can affect the water layer contained in the fillers, where hydrogen bonding silane should be established to connect to the matrix. All these mechanisms may weaken the interface between filler particles and matrix, which may lead to the reduction of filler size and decreased surface roughness [7]. Thus, evaluating the effects of fluoride therapy on composite resin microleakage is essential.

This study was conducted to evaluate the possible effects of pre-treatment topical fluoride therapy on marginal microleakage of composite resins in deciduous teeth. The results showed that pre-restorative topical fluoride treatment has no adverse effects on the marginal microleakage of composite restorations. Additionally, the measured microleakage was lower in the enamel margins than in dentinal ones.

Shabzender et al [6] evaluated the effect of pre- and post-topical fluoride therapy on marginal microleakage of aesthetic restorative materials (including composite resins and glass ionomers) in permanent teeth. They concluded that fluoride therapy with APF, pre- and post-placement of composite resin restorations, had no effect on marginal microleakage [6]. The results of the cited study are in accordance with that of the present study.

Tabari et al [7] also evaluated the effects of APF gel on the microleakage of fissure sealants and flowable composite resin restorations in permanent teeth. They revealed that the application of 1.23% APF gel had no significant effects on the microleakage of Tetric Flow® composite and Helioseal® fissure sealant; thus, this gel can be used routinely in the clinic [7]. Despite the fact that fluoride was applied after the restorative procedure in the cited study, the results were consistent with that of the present research; both studies showed that APF gel has no adverse effects on the microleakage of composite resin restorations.

Nystrom et al [12] (1989) investigated fluoride pre-treatment effects on the microleakage of a resin bonding agent. The results revealed no significant changes in the microleakage of enamel and dentinal walls [12], which is consistent with the result of the present research.

Composite resins are the first choice for aesthetic demands. Hybrid composites provide enhanced aesthetics and strength compared to traditional microfilled composites [9]. In this study, Filtek TM (Z250), a microhybrid composite, was used with a single-bond bonding agent.

Additionally, this study employed the “dye penetration” technique, because it is a simple, accessible, and financially reasonable method and the materials used are non-toxic and can be detected even at low concentrations [13].

Most in-vitro studies in the field of microleakage have been performed on Class V cavities. To simulate the oral cavity conditions, samples are usually tested under thermocycling and sometimes under mechanical loading [14].
In the present study, thermocycling was carried out. Barnes et al [15] (1994) showed no significant differences in the microleakage of enamel and cementum between buccal and lingual surfaces. The present study allowed us to evaluate both the lingual and buccal surfaces of each tooth. Von Fraunhofer et al [16] (2005) evaluated the effects of multiple uses of disposable diamond burs on restoration leakage. They showed that the reuse of disposable diamond burs can negatively affect microleakage. Cavities prepared with a new disposable diamond bur exhibit lower microleakage rates than cavities prepared with a reused bur [16]. Thus, we limited the use of each diamond bur to five teeth and each finishing disc to 10 teeth.

We tried to standardize the procedure of fluoride treatment with the routine clinical procedure. It is proven that in order to take the maximum advantage of preventive properties of a topical fluoride treatment, the teeth must be exposed to topical fluoride for at least 4 minutes [17-20]. After fluoride gel removal, all teeth were placed in artificial saliva to simulate the clinical conditions in which the patient is instructed not to rinse his/her mouth for at least 30 minutes.

The present study showed that 75% of the cavities had microleakage in dentinal margins. The differences in microleakage scores between occlusal and gingival walls of each group were also significant (P<0.05), similar to the results of previous studies [21-24]. There were no significant differences between gingival and occlusal microleakage in group 3 in which composite restorations were placed 30 minutes after fluoride therapy with 2% NaF (P=0.054).

The biological properties of dentin that may interfere with achieving a strong bond are as follows: high organic content [25], tubular structure [26], smear layer formation during cavity preparation [24], and intratubular fluid [27,28]. In previous studies, the effect of APF was evaluated, whereas in this study, neutral NaF gel, which is widely used in dental clinics nowadays, was also investigated. Furthermore, previous studies evaluated the microleakage of restorations placed immediately after fluoride therapy, while this study also examined the restorations placed two weeks after fluoride therapy.

According to the results of the present study, if topical use of fluoride does not have a negative effect on the bond strength and microleakage of composite restorations in deciduous teeth, fluoride therapy can be carried out before restoration placement. The clinical benefit of this approach is that both therapeutic procedures can be performed in one session. Moreover, fluoride therapy can increase enamel fluoride content and improve enamel strength. Thus, if parts of restoration were lost during the intervals between therapeutic sessions, the underlying enamel affected by fluoride would show higher resistance against caries due to the formation of calcium fluoride and gradual fluoride release.

Conclusion
Placement of composite resin restorations 30 minutes or 2 weeks after topical fluoride treatment by either 1.23% APF or 2% NaF does not affect marginal microleakage. Therefore, it seems that teeth can routinely and safely be restored with composite resins immediately after the application of topical fluoride.

References
1. Duke ES. Adhesion and its application with restorative materials. Dent Clin North Am. 1993 Jul;37(3):329-40.
2. Hilton TJ. Can modern restorative procedures and materials reliably seal cavities? In vitro investigations. Part 2. Am J Dent. 2002 Aug;15(4):279-89.
3. Moosavi H, Shabzendedar M, Shaibani V. Evaluation of the effect of pre- and post-topical fluoride treatment on marginal microleakage of resin composite and glass ionomer restorations. J Islamic Dent Assoc Iran. 2010 Fall;22(3):151-60.
4. Casamassimo P, Fields H, McTigue D, Nowak A. Pediatric Dentistry Infancy Through Adolescence. Philadelphia, PA: Elsevier Saunders, 5th Edition, 2012:279-81.
5. Dean JA, Avery DR, McDonald RE. McDonald
and Avery's Dentistry for the Child and Adolescent. Missouri, Mosby Elsevier, 10th Edition, 2015:179-82.

6. Shabzenderdar M, Moosavi H, Kebriaeef F, Daneshvar-Mozafari A. The effect of topical fluoride therapy on microleakage of tooth colored restorations. J Conserv Dent. 2011 Jul; 14(3):297-301.

7. Tabari M, Saffarcherati H, Soltankarimi V, Bani Ameri Z, Poorsattar Bejeh Mir A. Effect of Acidulated Phosphate Fluoride (APF) on the microleakage of composite flow and fissure sealant restorations. J Dent Med Tehran Univ Med Sci. 2011;24(4):238-44.

8. Derhami K, Coli P, Brannstrom M. Microleakage in Class 2 composite resin restorations. Oper Dent. 1995 May-Jun; 20(3):100-5.

9. Jones JA, Mash LK, Niessen LC. Restorative considerations for special needs patients. Dent Clin North Am. 1993 Jul;37(3):483-95.

10. Heymann H, Swift EJ, Ritter AV, Sturdevant CM. Sturdevant's Art and Science of Operative Dentistry. St. Louis, Mo: Elsevier/Mosby, 2013: 213-18.

11. Yap AU, Mok BY. Effects of professionally applied topical fluorides on surface hardness of composite-based restorative. Oper Dent. 2002 Nov-Dec;27(6):576-81.

12. Nystrom GP, Holtan JR, Olin PS, Douglas WH. Technical note: fluoride pre-treatment effects on microleakage of a resin bonding agent. Dent Mater. 1989 Sep;5(5):359-60.

13. Alan AH, Toh CG. Detection of microleakage around dental restorations: a review. Oper Dent. 1997 Jul-Aug;22(4):173-85.

14. Heymann H, Swift EJ, Ritter A. Sturdevant's Art and Science of Operative Dentistry. St. Louis, Mo: Elsevier/Mosby, 6th Edition, 2012: 161-6.

15. Barnes DM, McDonald NJ, Thompson VP, Blank LW, Shires PJ. Microleakage in facial and lingual Class 5 composite restorations: a comparison. Oper Dent. 1994 Jul-Aug; 19(4):133-7.

16. von Fraunhofer JA, Smith TA, Marshall KR. The effect of multiple uses of disposable diamond burs on restoration leakage. J Am Dent Assoc. 2005 Jan;136(1):53-7; quiz 90.

17. Marinho VC, Worthington HV, Walsh T, Chong LY. Fluoride gels for preventing dental caries in children and adolescents. Cochrane Database Syst Rev. 2015 Jun 15 (6):CD002280.

18. Jiang H, Bian Z, Tai BJ, Du MQ, Peng B. The effect of a bi-annual professional application of APF foam on dental caries increment in primary teeth: 24-month clinical trial. J Dent Res. 2005 Mar;84(3):265-8.

19. Jiang H, Tai B, Du M, Peng B. Effect of professional application of APF foam on caries reduction in permanent first molars in 6-7-year-old children: 24-month clinical trial. J Dent. 2005 Jul;33(6):469-73.

20. American Dental Association Council on Scientific Affairs. Professionally applied topical fluoride: evidence-based clinical recommendations. J Am Dent Assoc. 2006 Aug;137(8):1151-9.

21. Gordan VV, Vargas MA, Cobb DS, Deneyh GE. Evaluation of acidic primers in microleakage of Class 5 composite resin restorations. Oper Dent. 1998 Sep-Oct;23(5):244-9.

22. Sidhu SK, Henderson LJ. In vitro marginal leakage of cervical composite restorations lined with a light-cured glass ionomer. Oper Dent. 1992 Jan-Feb;17(1):7-12.

23. Fitchie JG, Puckett AD, Hembree JH, Williams M. Evaluation of a new dentinal bonding system. Quintessence Int. 1993 Jan;24(1):65-70.

24. Barkmeier WW, Cooley RL. Laboratory evaluation of adhesive systems. Oper Dent. 1992;Suppl 5:50-61.

25. Lopes GC, Baratieri LN, de Andrade MA, Vieira LC. Dental adhesion: present state of the art and future perspectives. Quintessence Int. 2002 Mar;33(3):213-24.

26. Pashley DH. Interactions of dental materials with dentin. Trans Acad Dent Mater. 1990;3:55-73.

27. Vargas MA, Cobb DS, Armstrong SR. Resin-dentin shear bond strength and interfacial ultrastructure with and without a hybrid layer. Oper Dent. 1997 Jul-Aug; 22(4): 159-66.

28. Li H, Burrow MF, Tyas MJ. Nanoleakage patterns of four dentin bonding systems. Dent Mater. 2000 Jan;16(1):48-56.