Effect of Resin Coating and Chlorhexidine on Microleakage of Two Resin Cements after Storage

F. Shafie 1, M. Doozandeh 2, A. Alavi 3

1 Associate Professor, Department of Operative Dentistry, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran
2 Postgraduate Student, Department of Operative Dentistry, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran
3 Assistant Professor, Operative Dentistry, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran

Abstract:
Objective: Evaluating the effect of resin coating and chlorhexidine on microleakage of two resin cements after water storage.

Materials and Methods: Standardized class V cavities were prepared on facial and lingual surfaces of one hundred twenty intact human molars with gingival margins placed 1 mm below the cemento-enamel junction. Indirect composite inlays were fabricated and the specimens were randomly assigned into 6 groups. In Groups 1 to 4, inlays were cemented with Panavia F2.0 cement. G1: according to the manufacturer’s instruction. G2: with light cured resin on the ED primer. G3: chlorhexidine application before priming. G4: with chlorhexidine application before priming and light cured resin on primer. G5: inlays were cemented with Nexus 2 resin cement. G6: chlorhexidine application after etching. Each group was divided into two subgroups based on the 24-hour and 6-month water storage time. After preparation for microleakage test, the teeth were sectioned and evaluated at both margins under a 20× stereomicroscope. Dye penetration was scored using 0-3 criteria. The data was analyzed using Kruskal-Wallis and complementary Dunn tests.

Results: There was significantly less leakage in G2 and G4 than the Panavia F2.0 control group at gingival margins after 6 months (P<0.05). There was no significant differences in leakage between G1 and G3 at both margins after 24 hours and 6 months storage. After 6 months, G6 revealed significantly less leakage than G5 at gingival margins (P=0.033). In general, gingival margins showed more leakage than occlusal margins.

Conclusion: Additionally, resin coating in self-etch (Panavia F2.0) and chlorhexidine application in etch-rinse (Nexus) resin cement reduced microleakage at gingival margins after storage.

Key Words: Resin Cements; Microleakage; Chlorhexidine; Resin Coating; Dental Leakage

2010; Vol. 7, No. 1
bond strength was reported [7-8]. However, there was a general trend toward higher bond strength causing less leakage [7]. Leakage tests are used to evaluate the marginal seal and the quality of the hybrid layer by assessing subsurface adaptation through evaluating dye penetration at the bonding interface [8].

Dual cure resin cements are used for luting indirect restorations to ensure optimal polymerization in deep areas. Adhesive systems are used to bond the resin cement to the tooth structure. These systems include both etch and rinse or self-etch [9,10]. In many studies, incompatibility between one-step self-etch/two-step etch and rinse adhesives and self or dual cure composites have been reported [11-13]. The presence of a high concentration of acidic, hydrophilic monomers and the lack of a hydrophobic resin layer in these adhesives, especially in one-step self-etch adhesives, contributed to adverse reactions between the acidic monomers and basic amines in the redox catalyst system and in the permeability of these adhesives [14,15]. Additionally, ternary redox initiators in the adhesives are used with resin cement for their optimal polymerization. Nevertheless, a relatively low bond strength of a self-etch cement, Panavia F, to hydrated dentin was reported, possibly related to adhesive permeability [9,16]. To overcome this permeability problem, application of a hydrophobic resin layer on ED primer before cementation with Panavia F or using a resin coating technique prior to taking an impression was suggested [9,17,18]. However, the latter method provides no freshly cut dentin as the ideal substrate for bonding during cementation.

Despite improvement in adhesive systems, the creation of a proper seal at the dentinal margin is still a problematic issue. Even if a complete seal is achieved with a preparation, the remaining bacteria can multiply and irritate the pulp and magnify the problems associated with microleakage.

Due to its antimicrobial effect, application of chlorhexidine (CH) to the cavity prior to its restoration has been recommended [19,20]. During the preparation and fabrication procedures of an indirect restoration, there is a greater possibility of bacterial contamination of the cavity. In addition, bacteria can remain in the smear layer when luting with self-etch resin cement. Thus, cavity disinfection prior to cementation is important. CH functions as a

| Resin Cement Manufacturer | Component Bath# | Enamel Dentin Pre-Treatment | Composite Pre-Treatment | Luting Agent Mixing |
|----------------------------|------------------|----------------------------|-------------------------|---------------------|
| Panavia F2.0, Kuraray Inc, Tokyo, Japan | ED primer A 00252, ED primer B 00129, Universal paste 00269, Catalyst paste 00053 | Mix one drop of each ED primer liquid A and B for 5s, air dry gently after 60s. | Apply K-etchant gel for 5s, rinse, air dry, mix one drop of each Clearfil SE primer and porcelain, Bond Activator for 5s. | Mix universal and catalyst paste for 20s, light cure for 20s, after removal excess cement, oxygen guard for 3 min. |
| Nexus 2, Kerr Co, Orange, USA | Optibond Solo Plus 2780278, Optibond Solo Plus activator 2864819 | Apply Kerr gel etchant (37% phosphoric acid) for 15s, rinse, air dry, mix one drop of Optibond Solo Plus and Optibond Solo Plus activator for 3s, apply to cavity, air dry and light cure for 20s. | Apply Kerr etchant gel for 15s, rinse, air dry, apply silane primer, air dry. | Mix base and catalyst paste for 10-20s, light cure for 40s. |
matrix metalloproteinase (MMP) inhibitor, apart from its antibacterial property, which may also prevent collagen degradation and disintegration of the bonding interface over time [21,22]. MMPs are a class of zinc- and calcium-dependent endopeptidases that remain in the dentin matrix during tooth development [21,23].

There is little information about the long-term sealing ability of one-step self-etch resin cement in combination with an additional resin layer or CH as an additional primer. Thus, the aim of this study was to test the null hypotheses that:

1) The addition of a resin layer to the self-etch cement, Panavia F2.0, has no effect on long-term dentinal microleakage.

2) The application of 2% CH prior to ED primer in Panavia F2.0 and after etching with an etch and rinse cement, Nexus 2, does not influence the marginal sealing of an indirect restoration after 6-months of storage.

MATERIALS AND METHODS

One hundred twenty extracted intact human molars were selected. All of the gingival remnants were removed and the crowns were thoroughly cleaned with prophylactic rotary instruments. The teeth were stored in 1% chloramine T solution at 4°C for one week and then stored in distilled water at 4°C for 3 months before use.

Standardized class V cavities (2 mm height, 4.5 mm length, 2 mm pulpal depth) were prepared on the facial and lingual surfaces of each tooth, with gingival margins 1 mm below the cemento-enamel junction using a straight diamond bur (# 878/d2, Teeskavan, Iran) in high speed handpiece under constant air-water spray. After each five preparations, the diamond burs were replaced.

Inlay fabrication and cementation: The cavities were lubricated with a water-soluble lubricating gel (Salem, Azardarmon, Iran), filled with one increment of indirect composite (Gradia, GC, Japan), and light cured for 40 seconds at 600 mW/cm² using a light curing unit (VIP junior, Bisco, USA). After primary curing, the composite inlays were removed from the cavities and the internal surfaces of the inlays were cured for an additional 40 seconds. Polymerization was completed in a Labo-Light LV III (GC, Japan). The inlay surface for bonding was sandblasted with 50 μm alumina particles (Dento-Prep, Denmark), and ultrasonically cleaned and dried. The prepared teeth were randomly assigned to six groups (n=40 cavi-

Table 2. Distribution of microleakage scores at occlusal and gingival margins after 24 hours (n=20).

| Group          | Score | Mean | SD  |
|----------------|-------|------|-----|
|                | 0     | 1    | 2   |
| Occlusal Margin|       |      |     |
| Panavia F2.0   | 17    | 3    | 0   |
| Panavia F2.0 + Liner | 20    | 0    | 0   |
| Panavia F2.0 + CH | 18    | 1    | 1   |
| Panavia F2.0 + CH+Liner | 17    | 2    | 1   |
| Nexus 2        | 16    | 3    | 1   |
| Nexus 2 + CH   | 17    | 2    | 1   |
|                | 3     | 4    |
|                | 0     | 3    |
|                | 0     | 3    |
|                | 0     | 3    |
|                | 0     | 3    |
|                | 0     | 3    |
|                | 0     | 3    |
|                | 0     | 3    |
|                | 0     | 5    |
|                | 0     | 5    |
|                | 0     | 5    |

2010; Vol. 7, No. 1
ties), corresponding to each luting protocol. Two resin cements were used and their manufacturer instructions are presented in Table 1. Before cementation, the intaglio surfaces of the composite inlays were prepared according to the manufacturer’s instructions for each cement (Table 1). The cavities were thoroughly cleaned and air-dried. Group 1; Panavia F2.0, (control): after application of ED primer 2, the inlays were cemented with Panavia F2.0 (Table 1) according to the manufacturer instructions and placed under a load of 500gr, simulating finger pressure, for one minute on the restorations. Light activation was performed for 60 seconds using a light curing unit (VIP junior).

Group 2; Panavia F2.0+liner: the cavity surface was primed with ED primer 2 and coated with a thin layer of HEMA free, unfilled hydrophobic resin (porcelain bonding resin, Bis-Co, USA, containing BisGMA, UDMA, TEGDMA) and light cured immediately for 20 seconds. The inlays were cemented with Panavia F2.0, similar to Group 1.

Group 3; CH+Panavia F2.0: 2% Chlorhexidine solution (Consepsis, Ultradent, USA) was applied to the cavities for 60 seconds and air dried for 10 seconds. The inlay was bonded similarly to Group 1.

Group 4: after applying CH similarly to group 3, the dentin surface was primed with ED primer 2, coated with a resin layer (porcelain bonding resin) and immediately light cured for 20 seconds. The inlays were then cemented with Panavia F2.0 similarly to Group 1.

Group 5; Nexus 2, (Control): after application of Optibond Solo Plus on the cavity surfaces, the inlays were cemented with dual cured cement, Nexus 2, (Kerr, USA) (Table 1) applying the same load as in Group 1. Group 6; CH+Nexus 2: after etching and rinsing, CH was applied to the cavities for 60 seconds and gently air dried for 5 seconds. The inlays were cemented similar to Group 5.

After cementation, the restorations were finished with carbide finishing burs (#448L, 012, Ultradent, USA) and polished using rubber impregnated abrasive points (Kerr, USA). Half of the specimens in each group were stored in distilled water at 37ºC for 24 hours and the other half were stored in distilled water at 37ºC for six months prior to leakage testing. During the storage period, the storage water was exchanged every week to prevent bacterial growth.

After each time interval, the specimens were blotted dry with a paper towel and the root apices were sealed with sticky wax. Two lay-

Table 3. Distribution of microleakage scores at occlusal and gingival margins after 6 months (n=20).

| Group                  | Score |     |     |     |     |     |
|------------------------|-------|-----|-----|-----|-----|-----|
|                        |       | 0   | 1   | 2   | 3   | Mean| SD  |
| Occlusal Margin        |       |     |     |     |     |     |
| Panavia F2.0           | 15    | 3   | 2   | 0   | 0.35| 0.671|
| Panavia F2.0 + Liner   | 16    | 2   | 2   | 0   | 0.30| 0.657|
| Panavia F2.0 + CH      | 16    | 1   | 3   | 0   | 0.35| 0.745|
| Panavia F2.0 + CH+Liner| 17    | 2   | 1   | 0   | 0.20| 0.523|
| Nexus 2                | 14    | 4   | 2   | 0   | 0.40| 0.681|
| Nexus 2 + CH           | 15    | 4   | 1   | 0   | 0.30| 0.571|
| Gingival Margin        |       |     |     |     |     |     |
| Panavia F2.0           | 5     | 3   | 5   | 7   | 1.70| 1.218|
| Panavia F2.0 + Liner   | 11    | 3   | 4   | 2   | 0.85| 1.089|
| Panavia F2.0 + CH      | 9     | 2   | 4   | 5   | 1.25| 1.293|
| Panavia F2.0 + CH+Liner| 13    | 3   | 2   | 2   | 0.65| 1.040|
| Nexus 2                | 0     | 2   | 8   | 10  | 2.40| 0.681|
| Nexus 2 + CH           | 3     | 7   | 4   | 6   | 1.65| 1.089|
ers of nail varnish were applied to all surfaces of the tooth except for 1 mm near the restoration margins. The teeth were immersed in a 1% solution of methylene blue dye for 24 hours at room temperature. After storage in the dye, the specimens were thoroughly rinsed with running water to remove excess dye. The specimens were then sectioned facio-lingually along the center of the inlay restoration, using a diamond saw (Leitz 1600, Germany) under water coolant. Dye penetration at the restoration/tooth interface was observed using a stereomicroscope at 20× magnification (Zeiss, Germany). Microleakage was determined for both the occlusal and gingival margins based on numerical criteria, as follows: 0=no leakage; 1=leakage up to one half the length of the cavity wall; 2=leakage along the full length of the cavity wall, not including the axial wall; 3=leakage along the axial wall.

Statistical analysis

Statistical analysis was performed using the non-parametric Kruskal-Wallis and complementary Dunn tests for the Panavia F2.0 groups (1-4). The Mann-Whitney U test was used for the Nexus 2 groups (5 and 6). The Mann-Whitney U test was also used for comparing the similar Panavia F2.0 and Nexus 2 groups (1 and 5).

A Wilcoxon signed rank test was used to compare the leakage between the occlusal and gingival margins in each group. All data were submitted for statistical analysis at α=0.05 level of significance.

RESULTS

The distribution of microleakage scores after 24 hours and 6 months at the occlusal and gingival margins are shown in Tables 2 and 3.

The Kraskal-Wallis test revealed no significant differences between Panavia F groups (1-4) at the occlusal margins after 24 hours and 6 months (P=0.36 and P=0.21, respectively) and at the occlusal margins after 24 hours, (P=0.89). However, a significant difference was observed among the Panavia F groups (1-4) at the gingival margin after 6 months. The complementary Dunn test revealed significant leakage differences between groups 1 and 2 (P=0.04) and groups 1 and 4 (P=0.03), such that the leakage of groups 2 and 4 was less than that of group 1.

The Mann-Whitney test showed that there was no significant difference between Panavia F groups regarding microleakage after 24 hours and 6 months, and it also showed that there was no significant difference between the two Nexus 2 groups at the occlusal margins after 24 hours (P=0.8) and 6 months (P=0.6) and at the gingival margins after 24 hours (P=0.76). However, a significant difference was observed in the leakage of gingival margins between Groups 5 and 6 after 6 months (P=0.033). There was no significant difference between Nexus 2 groups regarding leakage after 24 hours and 6 months. This test indicated that there was no difference at the occlusal and gingival margins between both the similar Panavia F2.0 and Nexus 2 groups at each time interval, although Nexus 2 had more leakage at the gingival margins after 6 months (P=0.09). A Wilcoxon signed rank test compared all occlusal margins versus gingival margins and indicated significantly more leakage at the gingival margins compared to the occlusal margins (P<0.05).

DISCUSSION

The longevity of indirect composite restorations is influenced by physico-mechanical properties of the restoration and its luting cement. Yet, the major factor in longevity is the bonding efficacy of the adhesives used in combination with the resin cement [24]. The adhesive systems can increase the bond strength and improve the seal between a resin cement and tooth structure [25]. Therefore, durable sealing has a great clinical importance. Indirect restorative procedures double the adhesive interfaces [10]. In the current evalua-
tion of microleakage of two cement complexes, leakage was observed at the tooth/cement interface. There was no leakage at the cement/restoration interface because of adequate surface treatment of the intaglio surface of the restoration.

In the present study, no significant difference was observed in the initial microleakage between the control group of Panavia F2.0 and Panavia F2.0 with an additional resin layer. Leakage in the two groups, especially when a liner was applied, was in the acceptable range (0 and 1).

Panavia F2.0 is a dual-cure resin cement, which is directly applied over the ED primed dentin without any hydrophobic resin bonding [9]. ED primer is a mild one-step self-etching primer, which can simultaneously demineralize dentin and infiltrate resin. The Panavia F2.0 system has sodium benzene sulphonate in the primer B composition and sodium aromatic sulphonate in the universal paste composition, ensuring adequate polymerization of the cement in the presence of an acidic monomer [9]. Nevertheless, Mac et al [16] reported low bond strength of Panavia F2.0 on flat, hydrated dentin without light curing. This may provide sufficient time for the acid-base reaction or adhesive permeability [16].

In the present study, with immediate light curing of the cement, rapid photo polymerization at the restoration margins was possible. Therefore, there was no sufficient time for any incompatibility. This adequate polymerization of the bonding interface at the margins may have resulted in the low leakage observed. However, the effect of the incompatibility of the cement adhesion to dentin at the deeper bonding interface beyond the cavity margins cannot be evaluated by means of the dye penetration technique. Especially, a thicker inlay (>3mm) would require a greater amount of chemical curing of resin cement to occur. In the study by Franco et al [26], the high bond strength of a dual-cure resin cement in combination with Prime and Bond 2.1 with a low pH was attributed to the quick initial hardening of the cement by light polymerization, which presented a protective function while the acid-base occurred more slowly. In addition, a higher conversion rate was reported for dual-cure resin cements with light curing compared to chemical curing [27]. In another study, low bond strength of Panavia F without light curing was found when compared to the addition of a resin layer. This reduced bond strength was related to increased permeability of the ED primer, manifested by the presence of blistering at the bonding interface [9].

In addition to the acidic, hydrophilic monomer, the high concentration of HEMA (30-50%) in ED primer can absorb water and form a hydrogel [28]. This additional pathway for water movement may lead to degradation of the bonding interface under long-term water storage [29]. Additionally, water evaporation from water-HEMA mixtures of primers is more difficult [30]. Incomplete polymerization due to remaining water and increased permeability in one-step self-etch adhesives might make these adhesives more susceptible to hydrolytic degradation over time. In the current study, even though the hydrophobic, unfilled, HEMA-free resin layer had no significant effect on decreasing gingival microleakage of Panavia F2.0 after 24 hours, this layer resulted in a significant decrease of microleakage when compared to the control group, with relatively constant microleakage observed after 6 months. Thus, the first part of null hypotheses was rejected.

More complete resin covering of collagen fibrils and residual spaces in the hybrid layer might have occurred due to a very low viscosity resin when compared to a relatively high viscosity of cement. This might have improved the quality of the hybrid layer and stability of marginal sealing over long-term. Moreover, the resin layer may also have contributed to the relief of polymerization stresses at the ad-
hesive interface in the unfavorable C-factor presented in the current study, as resin cement is used in cementing inlays [3,9].

Despite the susceptibility to hydrolytic degradation of Panavia F2.0/dentin interface in exposure to water, no significant difference in microleakage was observed after a 6-month period when compared to the 24-hour-period in the present study. This observation may be attributed to presence of MDP in both the ED primer and Panavia F2.0. Therefore, it was possible that the chemical bond between MDP and hydroxyapatite and hardly soluble calcium salts of MDP in water [31] had a protective effect on the hydrolytic degradation process, improving the long-term sealing of the inlay.

In the current study, the use of a resin layer may interfere with the fitting of the indirect inlay. However, based on TEM micrographs from Carvalho’s study [9], by adequate air thinning of the resin layer, the film thickness of the primer layer was increased by no more than 10 µm. Since the cement space in indirect restorations is 50-100 µm [32], a slight increase in the thickness of the primer layer may be partially compensated by a decrease in the thickness of the cement layer.

The other bond degradation mechanism involves deterioration of the dentin collagen matrix [21,35].

While the use of a low pH phosphoric acid during dentin etching might partially denature the MMPs, mild acids, such as those found in simplified etch and rinse adhesives, can activate new MMPs [23,36]. On the other hand, naked collagen fibrils at the base of the hybrid layer following incomplete resin penetration are susceptible to degradation by MMPs [37,38].

This degradation accounts for in-vivo and in-vitro observations of reduced integrity of the hybrid layer [35,39-41]. The collagenolytic activity of MMPs can be prevented through the use of MMP inhibitors, such as CH, which can preserve the long-term bond stability [21,22].

The use of CH with no effect on bond strength and microleakage of adhesives in direct restorations has been reported [42,43], although some studies reported that CH did have an adverse effect on bonding efficacy [44,45].

In the current study, CH had no effect on the initial microleakage of the two resin cements. This finding was in agreement with other bond strength studies of indirect restorations using an etch and rinse cement [46-48]. However, in a study by Hiraishi et al [48], the use of CH before Panavia F2.0 resulted in decreased bond strength and increased nanoleakage. Their explanation was that the adverse effect of CH may be attributed to the bonding of CH to loose, superficial apatites within the smear layer and the residual moisture of the CH solution, which might have interfered with the functioning of the ED primer. This latter effect has been confirmed by de Castro [42]; however, the dentin surface in the current study was relatively air-dried after application of CH.

In the current study, CH resulted in a considerable decrease in dentinal microleakage of the etch and rinse cement, Nexus 2, when compared to control group after 6-months of aging.

This finding may be attributed to the preservative effect of CH on the integrity of the hybrid layer. Thus, the second part of the null hypotheses was rejected for Nexus 2. The protective effect of CH on the bonding integrity of etch and rinse adhesives, such as Single Bond, has been reported [39-41].

In a study by Campos et al [49], the preservative effect of CH on bond strength of etch and rinse, and self-etch adhesives was reported during a 6-month aging period. This effect could be related to an increase of MMP activity by the self-etch adhesive [49-50].

However, in the present study, CH had no effect on the sealing ability of the self-etch cement, Panavia F2.0, after 6 months and the second part of the null hypotheses was con-
firmed for Panavia F2.0. Considering the similarity between depth of demineralization and resin infiltration, the presence of the remaining exposed collagen is not possible. In addition, due to the application of CH prior to ED primer on the smear layer covered dentin, collagen fibrils may not have been influenced by CH.

The observed positive effect of an added resin layer may be attributed to its protective effect on the collagen fibrils that were hydrolyzed by MMPs, because the resin layer can seal the matrix from the water that MMPs need for their action [21-50].

Further studies should be performed to validate the effect of CH on the long-term integrity of the hybrid layer in self-etch adhesives. Comparison of the leakage of two cements at both margins and at two time periods provided no significant difference, although there was a trend for more gingival leakage in the control group of Nexus after 6 months (P=0.09).

Gerdolle et al [51] reported less leakage of Panavia F than that presented with an etch and rinse cement (e.g. Variolink). In the current study, in all situations, gingival marginal leakage was considerably greater than enamel leakage. This finding was consistent with other resin cement leakage studies [4-6,51].

CONCLUSION

Within the limitations of this in vitro study, the below results were achieved:

1- An additional resin layer with Panavia F2.0 resulted in a significant reduction in gingival microleakage after a 6-month period of water storage.

2- The application of CH had no adverse effects on the initial microleakage of Panavia F2.0 and Nexus 2; however, after six months the use of CH resulted in a considerable reduction of leakage at the gingival margin in Nexus 2, while it had no effect on Panavia F2.0.

3- In general, enamel sealing in all groups was significantly better than dentin sealing.

REFERENCES

1- Krejci I, Lutz F, Reimer M, Heinzmann JL. Wear of ceramic inlays, their enamel antagonists, and luting cements. J Prosthet Dent 1993 Apr; 69(4):425-30.

2- Fuhrer N. Restoring posterior teeth with a novel indirect composite resin system. J Esthet Dent 1997;9(3):124-30.

3- Braga RR, Ferracane JL, Condron JR. Polymerization contraction stress in dual-cure cements and its effect on interfacial integrity of bonded inlays. J Dent 2002 Sep-Nov;30(7):333-40.

4- Hasegawa EA, Boyer DB, Chan DC. Microleakage of indirect composite inlays. Dent Mater 1989 Nov;5(6):388-91.

5- Hasanreisoglu U, Sonmez H, Uctasli S, Wilson HJ. Microleakage of direct and indirect inlay/onlay systems. J Oral Rehabil 1996 Jan;23(1):66-71.

6- Douglas WH, Fields RP, Fundingsland JA. A comparison between the microleakage of direct and indirect composite restorative systems. J Dent 2002;30:259-69.

7- 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.

8- Guzman-Ruiz S, Armstrong SR, Cobbs DS, Vargas MA. Association between microtensile bond strength and leakage in the indirect resin composite/dentin adhesively bonded joint. J Dent 2001 Feb;29(2):145-53.

9- Carvalho RM, Pegoraro TA, Tay FR, Pegoraro LF, Silva NR, Pashley DH. Adhesive permeability affects coupling of resin cements that utilise self-etching primers to dentin. J Dent 2004 Jan;32(1):55-65.

10- Hikita K, Van Meerbeek B, De Munck J, Ikeda T, Van Landuyt K, Maida T, et al. Bonding effectiveness of adhesive luting agents to enamel and dentin. Dent Mater 2007 Jan;23(1):71-80.

11- Sanares AM, Itthagarun A, King NM, Tay FR, Pashley DH. Adverse surface interactions between one-bottle light-cured adhesives and chemical-
cured composites. Dent Mater 2001 Nov;17(6):542-56.
12-Tay FR, Pashley DH, Suh BI, Carvalho RM, Itthagarun A. Single step adhesives are permeable membranes. J Dent 2002 Sep-Nov;30(7):371-82.
13-Cheong C, King NM, Pashley DH, Ferrari M, Toledano M, Tay FR. Incompatibility of self-etch adhesives with chemical/dual-cured composites: two-step vs one–step systems. Oper Dent 2003 Nov-Dec;28(6):747-55.
14-Tay FR, Pashley DH, Suh B, Carvalho R, Miller M. Single-step, self-etch adhesives behave as permeable membranes after polymerization. Part I. Bond strength and morphologic evidence. Am J Dent 2004 Aug;17(4):271-8.
15-Tay FR, Suh BI, Pashley DH, Prati C, Chaung SF, Li F. Factors contributing to the incompatibility between simplified –step adhesives and self-cured or Dual cured composites. Part II. Single –bottle, total-etch adhesive. J Adhes Dent 2003 Summer;5(2):91-105.
16-Mak YF, Lai SC, Cheung GS, Chan AW, Tay FR, Pashley DH. Micro-tensile bond testing of resin cements to dentin and an indirect resin composite. Dent Mater 2002 Dec;18(8):609-21.
17-Jayasooriya PR, Pereira PN, Nikaido T, Burrow MF, Tagami J. The effect of a “resin coating” on the interfacial adaptation of composite inlays. Oper Dent 2003 Jan-Feb;28(1):28-35.
18-Kitasako Y, Burrow MF, Nikaido T, Tagami J. Effect of resin-coating technique on dentin tensile bond strengths over 3 years. J Esthet Restor Dent 2002;14(2):115-22.
19-Fardal O, Turnbull RS. A review of the literature on use of chlorhexidine in dentistry. JADA J Am Dent Assoc 1986 Jun;112(6):863-9.
20-Sobral MA, Garone-Netto N, Luz MA, Santos AP. Prevention of postoperative sensitivity: a preliminary clinical trial. J Oral Rehabil 2005 Sep;32(9):661-8.
21-Pashley DH, Tay FR, Yiu C, Hashimoto M, Breschi L, Carvalho RM, et al. Collagen degradation by host-derived enzymes during aging. J Dent Res 2004 Mar;83(3):216-21.
22-Gendron R, Greiner D, Sorsa T, Mayrand D. Inhibition of the activities of matrix metalloproteinases 2, 8, and 9 by chlorhexidine. Clin Diagn Lab Immunol 1999 May;6(3):437-9.
23-Tjaderhane L, Larjava H, Sorsa T, Uitto VJ, Larmas M, Salo T. The activation and function of host matrix metalloproteinase in dentin matrix during breakdown in caries lesions. J Dent Res1998 Aug;77(8):1622-9.
24-Furukawa K, Inai N, Tagami J. The effects of luting resin bond to dentin on the strength of dentin supported by indirect resin composite. Dent Mater 2002 Mar;18(2):136-42.
25-Llena Puy MC, Forner Navarro L, Llopis LL, Fernandez A. Composite resin inlays: a study of marginal adaptation. Quintessence Int 1993 Jun;24(6):429-33.
26-Franco EB, Lopes LG, D’alpino PH, Pereira JC, Mondell RF, Navarro MF. Evaluation of compatibility between different types of adhesives and dual-cured resin cement. J Adhes Dent 2002 Winter;4(4):271-5.
27-Caughman WF, Chan DC, Rueggeberg FA. Curing potential of dual-polymerizable resin cements in simulated clinical situations. J Prostheth Dent 2001 Jul;86(1):101-6.
28-Beppas NA, Bures P, Leobandung W, Ichikawa H. Hydrogels in pharmaceutical formulations. Eur J Pharm Biopharm 2000 Jul;50(1):27-46.
29-Sauro S, Pashley DH, Montanari M, Chersoni S, Carvalho RM, Toledano M, et al. Effect of simulated pulpal pressure on dentin permeability and adhesion of self-etch adhesives. Dent Mater 2007 Jun;23(6):705-13.
30-Pashley EL, Zhang Y, Lockwood PE, Ruggeri FA, Pashley DH. Effects of HEMA on water evaporation from water-HEMA mixtures. Dent Mater 1998 Jan;14(1):6-10.
31-Choii KK, Condon JR, Ferracane JL. The effects of adhesive thickness on polymerization contraction stress of composite. J Dent Res 2000 Mar;79(3):812-7.
32-Toledano M, Osorio R, de Leonardi G, Rosales-Leal JI, Ceballos L, Cabrerizo-Vilchez MA. Influence of self-etching primer on the resin adhesion to enamel and dentin. Am J Dent 2001 Aug;14(4):
205-10.
33-Yoshida Y, Van Meerbeek B, Nakayama Y, Yoshioka M, Snauwaert J, Abe Y, et al. Adhesion to and decalcification of hydroxyapatite by carboxylic acids. J Dent Res 2001 Jun;80(6):39.
34-Molin MK, Karlsson SL, Kristiansen MS. Influence of film thickness on joint bond strength or a ceramic/resin composite joint. Dent Mater 1996 Jul;12(4):245-9.
35-Hashimoto M, Ohno H, Sano H, Kaga M, Ouchi H. In vitro degradation of resin-dentin bonds analyzed by microtensile bond test, scanning and transmission electron microscopy. Biomaterials 2003 Sep;24(21):3795-803.
36-Mazzoni A, Pashley DH, Nishitani Y, Brechin L, Mannello F, Tjäderhane L, et al. Reactivation of inactivated endogenous proteolytic activities in phosphoric acid-etched dentin by etch-and-rinse adhesives. Biomaterials 2006 Sep;27(25):4470-6.
37-Breschi L, Mazzoni A, Ruggeri A, Cademaro M, Di Lenarda R, De Stefano Dorigo E. Dental adhesion review: aging and stability of the bonded interface. Dent Mater 2008 Jan;24(1):90-101.
38-De Munck J, Van Meerbeek B, Yoshida Y, Inoue S, Suzuki K, Lambrechts P. Four-Year water degradation of total-etch adhesives bonded to dentin. J Dent Res 2003 Feb;82(2):136-40.
39-Brackett WW, Tay FR, Brackett MG, Dib A, Sword RJ, Pashley DH. The effect of chlorhexidine on dentin hybrid layers in vivo. Oper Dent 2007 Mar-Apr;32(2):107-11.
40-Hebling J, Pashley DH, Tjaderhane L, Tay FR. Chlorhexidine arrests subclinical degradation of dentin hybrid layers in vivo. J Dent Res 2005 Aug;84(8):741-6.
41-Carrilho MR, Carvalho RM, de Goes MF, di Hipolito V, Geraldeli S, Tay FR. Chlorhexidine preserves dentin bond in vitro. J Dent Res 2007 Jan;86(1):90-4.
42-de Castro FL, de Andrade MF, Duarte Junior SL, Vaz LG, Ahid FJ. Effect of 2% chlorhexidine on microtensile bond strength of composite to dentin. J Adhes Dent 2003 Summer;5(2):129-38.
43-Turkun M, Turkun LS, Kalender A. Effect of cavity disinfectants on the sealing ability of non-rinsing dentin-bonding resins. Quintessence Int 2004 Jun;35(6):469-76.
44-Gurgan S, Bolay S, Kiremitci A. Effect of disinfectant application method on the bond strength of composite to dentin. J Oral Rehabil 1999 Oct;26(10):836-84.
45-Tulunoglu O, Ayhan H, Olmez A, Bodur H. The effect of cavity disinfectants on microleakage in dentin bonding systems. J Clin Pediatr Dent 1998 Summer;22(4):299-305.
46-Turkun M, Cal E, Toman M, Toksavul S. Effects of dentin disinfectants on the shear bond strength of all-ceramics to dentin. Oper Dent 2005 Jul-Aug;30(4):435-66.
47-Soares CJ, Pereira CA, Pereira JC, Santana FR, do Prado CJ. Effect of chlorhexidine application on microtensile bond strength to dentin. Oper Dent 2008 Mar-Apr;32(2):183-8.
48-Hiraishi N, Yiu CK, King NM, Tay FR. Effect of 2% chlorhexidine on dentin microtensile bond strengths and nanoleakage of luting cements. J Dent 2009 Jun;37(6):440-8.
49-Campos EA, Correr GM, Leonardi DP, Barato-Filho F, Gonzalez CC, Zielak JC. Chlorhexidine diminishes the loss of bond strength over time under simulated pulpal pressure and thermomechanical stressing. J Dent 2009 Feb;37(2):108-14.
50-Nishitani Y, Yoshiyama M, Wadaonkar B, Breschi L, Mannello F, Mazzoni A. Activation of gelatinolytic/collagenolytic activity in dentin by self-etching adhesives. Eur J Oral Sci 2006 Apr;114(2):160-6.
51-Gerdolle DA, Mortier E, Loos-Ayav C, Jacquot B, Panigj MM. In vitro evaluation of microleakage of indirect composite inlays cemented with four luting agents. J Prosthodont 2005 Jun;93(6):563-70.