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

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Abstract

Purpose: This study evaluated the effect of resin coating and chlorhexidine (CH) on microleakage of two resin cements (Panavia F2.0, Nexus 2) after water storage.
Materials and Methods: Class V cavities were prepared on the facial and lingual surfaces of 120 intact human molars with gingival margins placed 1 mm below the cementoenamel junction. Indirect composite inlays were fabricated. The specimens were randomly assigned into six groups (n = 40). Indirect composite inlays (Gradia) were cemented as follows: Group 1 (control): inlays were cemented with Panavia F2.0 according to the manufacturer's instructions. Group 2: the ED-primed (ED Primer, Kuraray Dental, Tokyo, Japan) dentin was coated with a resin layer before cementation of the inlays with Panavia F2.0. Group 3: a 2% CH solution was applied before bonding with Panavia F2.0. Group 4: after CH application, the primed dentin was coated with a resin layer before cementation with Panavia F2.0. Group 5: (control) after applying Optibond Solo Plus, the inlays were cemented with Nexus 2. Group 6: after etching, CH was applied, and cementation was performed similarly to group 5. Half the specimens in each group were stored in distilled water for 24 hours, while the other half were stored in distilled water for 6 months. After storage, the teeth were placed in 1% methylene blue dye for 24 hours, sectioned, and evaluated under a 20× stereomicroscope. Dye penetration was scored using 0 to 3 criteria. Data were analyzed using nonparametric tests.
Results: Resin coating of ED primer for Panavia F2.0 significantly reduced microleakage at the gingival margins after 6 months ($p < 0.05$). CH application in Panavia F2.0 did not lead to a significant difference in the microleakage at both margins, after 24 hours and 6 months ($p > 0.05$). The application of CH showed significantly less microleakage than that of the control group at the gingival margins of Nexus 2 after 6 months. In general, gingival margins showed more microleakage than occlusal margins.
Conclusion: An additional resin layer applied to a self-etch cement can improve long-term dentinal sealing for indirect restorations, while CH cannot; however, CH reduces gingival microleakage in an etch-and-rinse cement after aging.

Indirect composite restorations are increasingly used in restorative dentistry for inlay, onlay, veneer, and crown restorations due to improved mechanical properties and controlled polymerization shrinkage stresses.[1] Polymerization shrinkage is limited to a thin layer of resin cement used to lute the restoration;[2] however, this polymerization stress due to the lack of unbonded surfaces may disrupt the bond between the resin cement and the cavity walls or the inlay walls. The resin cement shrinkage may result in gaps or voids at the cavity (cement or inlay) cement interfaces, leading to microleakage.[3–6]

In vitro bond strength and microleakage studies are employed to examine the strength and integrity of the marginal seal of indirect restorations. The literature reports no consensus on the correlation between microleakage and bond strength;[7,8] however, a general trend toward higher bond strength having less microleakage exists.[7] Microleakage 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]

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Dual-cure resin cements are used for luting indirect restorations (such as indirect composites) to ensure optimal polymerization in deep areas. Etch-and-rinse and self-etch adhesive systems are used to bond the resin cement to the tooth structure.[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 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 the problem of permeability, the 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] Despite improvement in adhesive systems, the creation of a proper seal at the dentinal margin is still a problematic issue. Due to its antimicrobial effect, the 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 may remain in the smear layer when luting with self-etch resin cement. Thus, cavity disinfection prior to cementation is important. Apart from its antibacterial properties, CH functions as a matrix metalloproteinase (MMP) inhibitor, 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 present study was to test the null hypotheses that:

1. The addition of a resin layer to a self-etch cement, Panavia F2.0, has no effect on long-term dentinal microleakage.
2. The application of 2% CH prior to ED primer II in Panavia F2.0 does not influence the marginal sealing of an indirect restoration after 6 months of storage.
3. The application of 2% CH after etching with an etch-and-rinse cement, Nexus 2, does not have any effect on marginal sealing after aging.

Materials and methods

One hundred twenty extracted intact human molars were selected. All 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 1 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 long, 2-mm pulpal depth) were prepared on the facial and lingual surfaces of each tooth, with gingival margins 1 mm below the cementoenamel junction using a straight diamond bur (#878/d2, Teeskavan, Iran) in a high-speed handpiece under constant air–water spray. After every five preparations, the diamond burs were replaced.

Inlay fabrication and cementation

The cavities were lubricated with a water-soluble lubricating gel (Salem, Azardarman, Iran), filled with one increment of indirect composite (Gradia, GC, Tokyo, Japan), and light cured for 40 seconds at 600 mW/cm^2, using a light-curing unit (VIP junior, Bisco, Schaumburg, IL). 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) for 3 minutes. The inlay surface for bonding was sandblasted with 50 μm alumina particles (Dento-Prep, Ronvig, Denmark), ultrasonically cleaned, and dried. The prepared teeth were randomly assigned to six groups (n = 40 cavities), corresponding to each luting protocol. Two resin cements were used, with their manufacturer instructions presented in Table 1. The six tested groups are summarized in Table 2.

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 II, the inlays were cemented with Panavia F2.0 (Table 1) according to the manufacturer's instructions and placed under a 500-g load, simulating finger pressure, for 1 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 II and coated with a thin layer of HEMA-free, unfilled hydrophobic resin (porcelain bonding resin, Bisco, 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% CH solution (Consepsis, Ultradent, South Jordan, UT) was applied to the cavities for 60 seconds and air dried for 10 seconds. The inlay was bonded similarly to Group 1.

Group 4, CH + ED primer II + Liner + Panavia F2.0: After applying CH similar to group 3, the dentin surface was primed with ED primer II, 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, Orange, CA) (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) and polished using

Table 1: Resin cement systems used and their application procedures

Resin cement, manufacturer	Component, batch#	Enamel, dentin pretreatment	Composite pretreatment	Luting agent mixing
Panavia-F2.0 Kuraray Inc, Tokyo, Japan	ED primer, II, A00252 ED primer II, B00129 Universal paste, 00269 Catalyst paste, 00053	Mix one drop of each ED primer liquid A and B for 5 seconds, air dry gently after 60 seconds	Apply K-etchant gel for 5 seconds, rinse, air dry, mix one drop each Clearfil SE primer and Porcelain Bond Activator for 5 seconds, apply.	Mix universal and catalyst paste for 20 seconds, light cure for 20 seconds, after removal of excess cement, apply oxyguard for 3 min.
Nexus 2 Kerr Co, Orange,CA	Optibond Solo Plus, 2780278 Optibond Solo Plus activator, 2864819 Base paste, 2858971 Catalyst paste, 2858391	Apply Kerr gel etchtant (37% phosphoric acid) for 15 seconds, rinse, air dry, mix one drop of Optibond Solo Plus and Optibond Solo Plus activator for 3 seconds, apply to cavity, air dry, and light cure for 20 seconds.	Apply Kerr gel etchant for 15 seconds, rinse, air dry, apply silane primer, air dry.	Mix base and catalyst past for 10 to 20 seconds, light cure for 40 seconds.

rubber-impregnated abrasive points (Kerr). Half the specimens in each group were stored in distilled water at 37°C for 24 hours, and the other half were stored in distilled water at 37°C for 6 months prior to microleakage testing. During the storage period, the storage water was exchanged every week to prevent bacterial growth.

Microleakage assessment

After each time interval, the specimens were blotted dry with a paper towel, and the root apexes were sealed with sticky wax. Two layers 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, Munich, Germany) under water coolant. Dye penetration at the restoration/tooth interface was observed using a stereomicroscope at 20× magnification (Zeiss, Oberkochen, Germany). Microleakage was determined for both the occlusal and gingival margins based on numerical criteria, as follows: 0 = no microleakage; 1 = microleakage up to one half the length of the cavity wall; 2 = microleakage along the full length of the cavity wall, not including the axial wall; 3 = microleakage along the axial wall.

Table 2 Groups tested

Group 1	ED primer II ⇒ Panavia F2.0
Group 2	ED primer II ⇒ Resin liner ⇒ Panavia F2.0
Group 3	CH ⇒ ED primer II ⇒ Panavia F2.0
Group 4	CH ⇒ ED primer II ⇒ Resin liner ⇒ Panavia F2.0
Group 5	Etching ⇒ Optibond Solo Plus + activator ⇒ Nexus 2
Group 6	Etcing ⇒ CH ⇒ Optibond Solo Plus + activator ⇒ Nexus 2

CH = Chlorhexidine.

Statistical analysis

Since microleakage was measured as an ordinal scale, statistical analysis was performed using the nonparametric Kruskal-Wallis and, if applicable, complementary Dunn tests for pairwise comparison among the Panavia F2.0 groups (1 to 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 microleakage between the occlusal and gingival margins in each group ($p < 0.001$). All data were submitted for statistical analysis at the $p < 0.05$ 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 3 and 4. The Kruskal-Wallis test revealed no significant differences between Panavia F2.0 groups (1 to 4) at the occlusal margins after 24 hours and 6 months and at the gingival margins after 24 hours ($p > 0.05$); however, a significant difference was observed among the Panavia F2.0 groups (1 to 4) at the gingival margin after 6 months. The complementary Dunn test revealed that the gingival microleakage of groups 2 and 4 was significantly less than that of group 1 ($p < 0.05$), indicating the beneficial effect of resin layer.

The Mann-Whitney U test showed that there was no significant difference in microleakage of each group of Panavia F2.0 between 24 hours and 6 months ($p > 0.05$). The Mann-Whitney U test showed that there was no significant difference in the two Nexus groups at the occlusal margins after 24 hours and 6 months and at the gingival margins after 24 hours ($p > 0.05$); however, a significant difference was observed in the microleakage of gingival margins between groups 5 and 6 after 6 months ($p < 0.05$), demonstrating that CH application resulted in lower microleakage. There was no significant difference in microleakage of the Nexus 2 group between 24 hours and 6 months. The Mann-Whitney U test indicated there was

Table 3 Distribution of microleakage scores at the occlusal and gingival margins after 24 hours (n = 20)

	Score				
Group	0	1	2	3	Median
Occlusal margin					
Group 1	17	3	0	0	0
Group 2	20	0	0	0	0
Group 3	18	1	1	0	0
Group 4	17	2	1	0	0
Group 5	16	3	1	0	0
Group 6	17	2	1	0	0
Gingival margin					
Group 1	8	5	4	3	1
Group 2	12	4	3	1	0
Group 3	8	6	3	3	1
Group 4	14	2	2	2	0
Group 5	5	3	5	7	2
Group 6	5	5	5	5	1.5

no difference at the occlusal and gingival margins between both the similar Panavia F2.0 and Nexus 2 groups at each time interval. Wilcoxon signed rank test compared all occlusal margins versus gingival margins and indicated significantly more microleakage at the gingival margins than at the occlusal margins ($p < 0.001$).

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 great clinical importance.

Table 4 Distribution of microleakage scores at the occlusal and gingival margins after 6 months (n = 20)

	Score				
Group	0	1	2	3	Median
Occlusal margin					
Group 1	15	3	2	0	0
Group 2	16	2	2	0	0
Group 3	16	1	3	0	0
Group 4	17	2	1	0	0
Group 5	14	4	2	0	0
Group 6	15	4	1	0	0
Gingival margin					
Group 1	5	3	5	7	2
Group 2	11	3	4	2	0
Group 3	9	2	4	5	1
Group 4	13	3	2	2	0
Group 5	0	2	8	10	2.5
Group 6	3	7	4	6	1.5

Brackets indicate significantly different group pairs ($p < 0.05$).

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

In the present study, with immediate light curing of the cement, rapid photo polymerization at the restoration margins was possible. Therefore, there was not sufficient time for any incompatibility in the Panavia F2.0 system. This adequate polymerization of the bonding interface at the margins may have resulted in the low microleakage 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 (>3 mm) would require a greater amount of chemical curing of resin cement to occur. In a study by Franco et al,[26] the high bond strength of a dual-cure resin cement in combination with Prime & 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. Thus, immediate light polymerization might prevent any incompatibility of the resin cement-acidic adhesive. In addition, a higher conversion rate was reported for dual-cure resin cements with light curing compared to chemical curing.[27]

In addition to the acidic, hydrophilic monomer, the high concentration of HEMA (30% to 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 in microleakage when compared to the control group, with relatively constant microleakage observed after 6 months. Thus, the first part of the null hypotheses was rejected.

The additional light-cured resin layer might improve the rate and extent of polymerization of a self-etching primer due to additional free radicals.[9] This might have decreased the permeability of the adhesive, resulting in an improvement of the long-term durability of this adhesive interface.[31]

More complete resin covering of collagen fibrils and residual spaces in the hybrid layer might have occurred due to a very low viscosity resin (porcelain-bonding resin) when compared to the relatively high viscosity of cement (Panavia F2.0). This might have improved the quality of the hybrid layer and stability of marginal sealing over the long term. Moreover, the unfilled resin layer with a low modulus of elasticity may also have contributed to the relief of polymerization stresses at the adhesive interface, as resin cement is used in cementing inlays.[3,9,32]

Despite the susceptibility to hydrolytic degradation of the 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 the presence of MDP in both ED primer II 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[33] 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 have interfered with the fitting of the indirect inlay; however, on the basis of transmission electron microscopy (TEM) micrographs from Carvalho et al's study,[9] by adequate air thinning of the resin layer, the film thickness of the primer layer was no more than 10 μm. Since the cement space in indirect restorations is 50 to 100 μm,[34] a slight increase in the thickness of the primer layer may be partially compensated for by a decrease in the thickness of the cement layer.

It has been suggested that a resin layer can be used after cavity preparation and before taking an impression to reduce the fitting problem.[17] Additionally, the beneficial effect of this resin-coating technique in bonding durability has been reported.[18,35,36] In previous studies, different adhesives with low-viscosity resins were used, and only Panavia F2.0 was used for cementation without ED primer. The current study evaluated the effect of an additional resin layer on the complete Panavia F2.0 system.

The other bond degradation mechanism involves deterioration of the dentin collagen matrix.[21,37] 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.[23,38] On the other hand, naked collagen fibrils at the base of the hybrid layer following incomplete resin penetration are susceptible to degradation by MMPs.[39,40] This degradation accounts for in vivo and in vitro observations of reduced integrity of the hybrid layer.[38,41–43] 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 had no effect on bond strength and microleakage of adhesives in direct restorations;[44,45] however, other studies have reported that CH had an adverse effect on bonding efficacy.[46,47] 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 bonding system;[48,49] however, in a study by Hiraishi et al,[50] 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 II. This latter effect is a confirmation of a previous report by de Castro et al;[44] however, the dentin surface in the current study was relatively air-dried after the application of CH.

In the current study, CH resulted in a considerably lower amount of dentinal microleakage of the etch-and-rinse cement, Nexus 2, when compared to the 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 through preventing collagen hydrolysis. Thus, the third part of the null hypothesis was rejected. The protective effect of CH on the bonding integrity of etch-and-rinse adhesives, such as Single Bond, was reported.[41–45]

In a study by Campos et al,[51] the preservative effect of CH on the 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;[51,52] 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 confirmed. Considering the similarity between depth of demineralization and resin infiltration, the presence of the remaining exposed collagen is not possible. Also, due to the application of CH prior to ED primer II 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.[21,52] 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 microleakage of two cements at both margins and at two time periods provided no significant difference, although there was a trend for more gingival microleakage in the Nexus control group after 6 months ($p = 0.09$). Gerdolle et al[53] reported less microleakage of Panavia F than that presented with an etch-and-rinse cement (Variolink). In the current study, in all situations, gingival marginal microleakage was considerably greater than enamel microleakage. This finding was consistent with other microleakage studies of resin cements.[4–6,53] With respect to the minimal effect of artificial aging methods, such as water storage, on microleakage tests as compared to bond strength tests,[54] more precise bond strength comparisons should be conducted in this field.

Conclusions

Based on the results of this in vitro study:

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.

3. Sealing ability of Panavia F2.0 was not affected by CH application after 6 month.
4. After 6 months, the use of CH resulted in a considerable reduction of microleakage at the gingival margin in Nexus 2, while it had no effect on Panavia F2.0.
5. In general, enamel sealing in all groups was significantly better than dentinal sealing.

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