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Microleakage of three different combinations of adhesive and composite resins

E. FERRARI CAGIDIACO, D. KARAFILI, G. VERNIANI, G. ZUCCA, M. FERRARI*

Department of Prosthodontics and Dental Biomaterials, School of Dental Medicine, University of Siena, Italy
*MD, DMD, PhD, Dean, Chair and Professor

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ABSTRACT

Aims The aim of the present *in vitro* study was to evaluate the influence of 3 adhesive systems on microleakage of direct composite restorations with proximal margins under the cement-enamel junction (CEJ) and in the enamel.

Materials and methods In 30 extracted molars standardized MOD (mesio-occlusal-distal) cavities were prepared with a proximal margin located 1 mm under the cement-enamel junction and another one in the enamel, and subsequently randomly divided in 3 groups of 10 using 3 different adhesives and the same composite: Flowable (G-aenial Universal Injectable, GC) + universal adhesive (G2-Bond Universal, GC) with selective-etch technique (Group 1); Flowable (G-aenial Universal Injectable, GC) + self-etch adhesive (Clearfil SE Bond 2, Kuraray) with 2-step technique (Group 2); Flowable (G-aenial Universal Injectable, GC) + total-etch adhesive (Optibond FL, Kerr) with 3-step technique (Group 3). Samples were tested for microleakage using silver nitrate and infiltration was classified in 5 levels. The differences in microleakage were statistically evaluated with significance set at $p<0.05$.

Results In the margin located in the enamel, group 1 showed an average of 0 microleakage, group 2 an average of 0.2 and group 3 an average of 0.1. In the margin located in the dentin, group 1 showed an average score of 1.1, group 2 of 2.15 and group 3 of 1.25. No statistically significant difference was found in the enamel.

Conclusion The combination of adhesive G2-Bond Universal and Optibond FL showed the highest sealing ability both in margins located in the enamel and margins located in the dentin. The adhesive interface in the enamel produced a very good seal, while the adhesive interface in the dentin showed varying degrees of microleakage in all groups.

KEYWORDS Microleakage; Adhesives; Composite resins.

INTRODUCTION

Conventional amalgam restorations have been replaced by the use of adhesive resin materials that have improved the aesthetics of dental restorations in the posterior region (1-2) and are also used in the reconstruction of the cervical margin. Moreover, minimally invasive adhesive restorations protect the intact tooth structure without sacrificing it for mechanical retention (3).

Optimal isolation of the restoration in the subgingival margin is crucial for long term success. The relocation of the cervical margin can be performed with hybrid or fluid composites, after isolation with rubber dam, positioning of the metal matrix and the interproximal wedge. Isolation of the field with rubber dam improves the quality of the treatment and protects the patient from accidentally swallowing irritating liquids and instruments. It also reduces the risk of accidental damage to soft tissues with burs or sharp instruments caused by sudden movements.

The absence of enamel at the cervical margin creates areas of weak adhesion. Adhesion of dentin is not as stable as that of enamel (4) and is associated with increased risks of bacterial penetration, hypersensitivity and secondary caries. In addition, the composite resin material and adhesive interface at the level of the cervical margin reconstruction degrade under occlusal loading, thus allowing penetration of bacterial biofilm in the dentin restoration margin and, possibly, faster development of secondary caries *in vivo*. Therefore, since enamel and dentin are two different substrates, the bonding of the restoration will also be different.

The adhesion process involves two steps.

1. Preparation of the site, with removal of calcium phosphates and expansion of both enamel and dentin micropores.
2. Infiltration hybridization and resin polymerization.

This procedure aims to create an infiltration in the microporosity of the tooth and a mechanical interconnection: the first is based on interconnection by the hydrophilic resin, while the latter is achieved through mechanical addition between the functional monomers and the dental substrate. All this leads to the formation of the hybrid layer, i.e. an interdiffusion between the resin and the tooth. This procedure is aimed at providing a seal of the restoration that is maintained over time.

The hybrid layer varies with the restorative materials used. Some authors have suggested that flowable composites are the first choice of materials for lifting the margin in deep cavities (5-9). Others support the use of flowable or of resinous composites (6,7,8) or a combination of both if more material is needed. However, there is no consensus on the material of choice nor on the application technique for direct restorations at both the enamel and dentin levels.

The aim of this *in vitro* study was to evaluate the influence of different adhesive systems (three adhesive systems in combination with a flowable resin composite) on the microleakage of direct composite restorations with one proximal margin placed under the cement-enamel junction (CEJ) and the other in the enamel. Moreover, the microleakage of silver nitrate at the enamel level and at the dentin level was evaluated through the use of microscope.

MATERIALS AND METHODS

The present in vitro study was conducted on 30 intact human molars, extracted for dental reasons after an informed consent was signed by all patients.

The teeth were mechanically cleaned with scalers and brush mounted on a micromotor with prophylaxis paste (Nupro, Dentsply). Subsequently, in each tooth, standardized MOD (mesio-occlusal-distal) cavities were prepared, under water cooling, with diamond burs for proximal margins placed 1 mm below the CEJ and a round bur for margins at the dentin level.

For the study, three different universal adhesives (G2-Bond Universal, GC; Clearfil SE Bond 2, Kuraray; Optibond FL, Kerr) were selected for use in combination with a flowable composite (G-aenial Universal Injectable, GC).

Teeth were then randomly divided into 3 groups (Table 1) and restorations were performed using incremental technique as follows.

- Group 1: restoration with flowable composite (G-aenial Universal Injectable, GC) and universal adhesive (G2-Bond Universal, GC) with selective-etch mode.
- Group 2: restoration with flowable composite (G-aenial Universal Injectable, GC) and self-etch adhesive (Clearfil SE Bond 2, Kuraray) in 2-step mode.
- Group 3: restoration with flowable composite (G-aenial Universal Injectable, GC) and total-etch adhesive (Optibond FL, Kerr) in 3-step mode.

	Group 1	Group 2	Group 3
Adhesive	G2-Bond Universal	Clearfil SE Bond 2	Optibond FL
Composite	G-aenial Universal Injectable	G-aenial Universal Injectable	G-aenial Universal Injectable

TABLE 1 Materials used for the restorations in the experimental groups.

Subsequently, all surfaces of the 30 teeth were covered with a common nail polish, and 1 mm was left exposed around the area of the adhesive interfaces between the tooth and the restoration (Fig. 1).

Each tooth was placed in a test tube with a diluted solution of ammonia silver nitrate (ammonia silver nitrate and distilled water in a ratio of 1:4) filtered with a millipore filter mounted on a syringe. After 24 hours the samples were rinsed three times in distilled water for 10 minutes.

Teeth were divided into 3 groups, i.e. 10 teeth per group, and the polish, previously applied, was removed using acetone. Then each tooth was put back in the test tube and immersed in a diluted photo-developing solution (developer and distilled water in a ratio of 1:10) for 8 hours.

Samples were then rinsed three times in distilled water for 10 minutes.

Each tooth was dried and embedded in transparent self-curing acrylic resin.

Subsequently the teeth were cut along their longitudinal axis and perpendicular to the proximal margin with a low speed diamond disc under water cooling in four to five slices about 1 mm thick (Fig. 2).

Samples were then examined with a digital microscope at x1, x3, x6 magnification. The infiltration level (i.e. amount of silver nitrate present along the interface) was evaluated and scored as follows (Fig. 3).

- 0: no leakage.
- 1: 0% to 20% of gingival floor interface leakage.

FIG. 1 Preliminary tooth preparation.

FIG. 2 Slicing of samples.

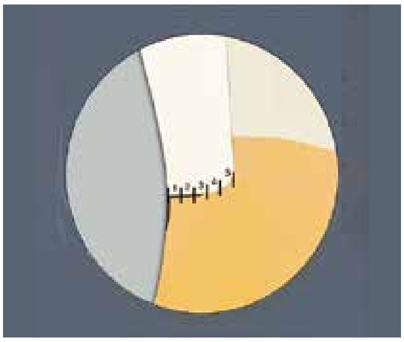

FIG. 3 The scoring pattern.

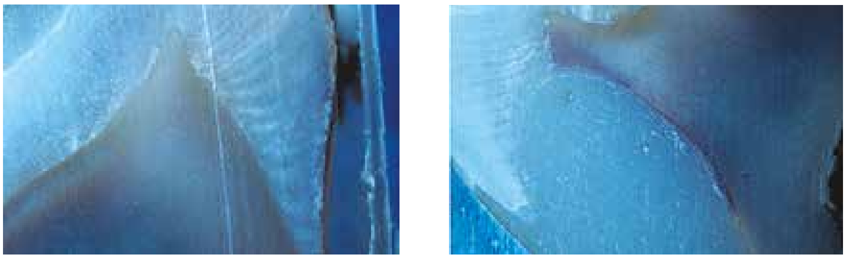

FIG. 4 Optical microscope images of samples from group 1.

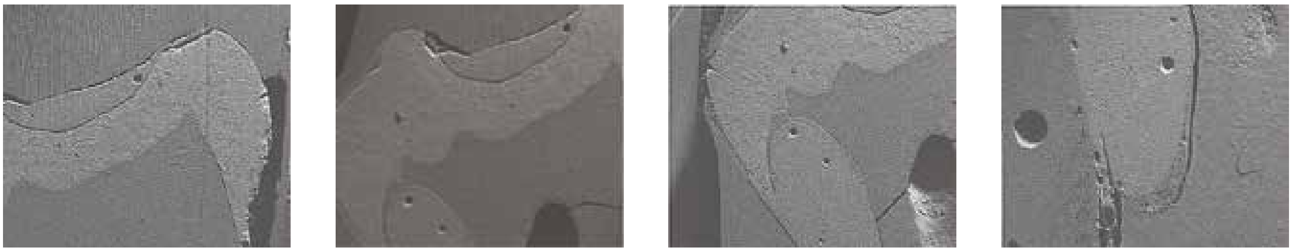

FIG. 5 SEM microscope images of samples from group 1.

- 2: 20% to 40% of gingival floor interface leakage.
- 3: 40% to 60% of gingival floor interface leakage.
- 4: 60% to 80% of gingival floor interface leakage.
- 5: 80% to 100% of gingival floor interface leakage.

This was observed under the microscope both at the dentin and at the enamel levels on all the sites of each single tooth (Fig. 4, 5). Also, some samples of each group were randomly selected and observed under SEM (Jeol, Tokyo, Japan).

The differences in microleakage were statistically evaluated with significance set at $p<0.05$. For statistical analysis the Kruskal-Wallis analysis of variance by rank and the Wilcoxon signed-ranks test were used.

RESULTS

Microleakage of direct restorations using three different combinations of adhesives with different viscosities and composite was evaluated at different interfaces: proximal margin under the CEJ and in the enamel.

The results are reported in table 2 and represent the average of the scores of each section of each sample, divided by group, both at the level of the enamel and at the level of the dentin.

The best seal was obtained at the level of the enamel with the universal adhesive (Table 2).

Group	Enamel score	Dentin score
1	0	1,1
2	0,2	2,15
3	0,1	1,25

TABLE 2 Scores of the three groups.

Statistical analysis

The Kruskal-Wallis analysis of variance by rank was used to assess statistical significance of differences in microleakage scores among the 3 experimental groups at the enamel and dentin levels.
The Wilcoxon signed-rank test was used to assess the statistical significance of differences in microleakage scores in enamel and dentin within each experimental group.
In all tests the level of statistical significance was set at $p < 0.05$ (Table 3, 4).
The Kruskal-Wallis analysis of variance by rank did not reveal any statistically significant difference among the 3 experimental groups both at the enamel ($p = 0.1$) and at the dentin level ($p = 0.057$), so the materials used have very similar results.
Descriptive statistics of microleakage scores recorded on enamel and dentin within each of the 3 experimental groups are reported in Tables 5, 6 and 7.
Wilcoxon's signed-rank test revealed that Group 1 had significantly higher microleakage scores in dentin than in enamel ($p < 0.001$) (Table 5).
Wilcoxon's signed-rank test revealed that Group 2 had significantly higher microleakage scores in dentin than in enamel ($p < 0.001$) (Table 6).
Wilcoxon's signed-rank test revealed that Group 3 had significantly higher microleakage scores in dentin than in enamel ($p = 0.002$) (Table 7).
The Wilcoxon signed rank test, used to analyze the statistical significance of the differences in the microleakage scores in enamel and dentin within each experimental group, showed in all three groups a greater microleakage at the dentin level.

Group	N	Median	Interquartile range
1	20	0	0-0
2	20	0	0-0
3	20	0	0-0

TABLE 3 Descriptive statistics of microleakage scores recorded in enamel in the 3 experimental groups.

Group	N	Median	Interquartile range
1	40	1	0-2
2	45	3	1-3
3	42	0,5	0-3

TABLE 4 Descriptive statistics of microleakage scores recorded in dentin in the 3 experimental groups.

Substrate	N	Median	Interquartile range
Enamel	20	0	0-0
Dentin	20	1	0-2

TABLE 5 Descriptive statistics of microleakage scores recorded in enamel and dentin in Group 1.

Substrate	N	Median	Interquartile range
Enamel	20	0	0-0
Dentin	20	3	1-3

TABLE 6 Descriptive statistics of microleakage scores recorded in enamel and dentin in Group 2.

Substrate	N	Median	Interquartile range
Enamel	20	0	0-0
Dentin	20	0,5	0-3

TABLE 7 Descriptive statistics of microleakage scores recorded in enamel and dentin in Group 3.

DISCUSSION

In the present *in vitro* study, it was observed that microleakage is almost absent in the enamel interface. This result is most likely due to the fact that etched and milled enamel prisms ensure effective micromechanical interdigitation, which prevents adhesive and cohesive fracture at the enamel-adhesive interface (10,11).
Microleakage at the level of the enamel is lower than that of the dentin due to its chemical and physical characteristics. In fact, as reported in the literature, adhesion to enamel is a safe and effective procedure. Owing to the inorganic composition of the enamel, etching procedures dissolve the prismatic and interprismatic substance thus creating irregularities through which the resin can flow and, after polymerization, achieve a stable mechanical interdigitation (12).
With respect to adhesives, our data show that the best marginal seal, on both enamel and dentin, is obtained with G2-Bond Universal and OptiBond FL adhesives.
Universal adhesives are the latest generation of adhesive systems and are less technique sensitive (13). In addition, the application of universal adhesive to dentin reduces the risk of excessive etching and ensures that the dentin

substrate is not too dry or too wet (14,15). Universal adhesive systems have shown promising results so far (16-18).
On the basis of the present results, it can be stated that the seal on enamel is very reliable with each of the adhesives used, especially with universal ones. On the contrary, dentin gave lower results with all adhesives, which means that microleakage is higher at the dentin level. In fact, dentin is a naturally moist substrate and therefore intrinsically hydrophilic. Dentin composition may vary depending on the tooth area: there are distinct differences in the depth of demineralization of the dentin and in the degree of penetration of the adhesive between the proximal surfaces and the gingival margin. The dentin at the gingival margin is less mineralized than the dentin in the proximal wall, while diameter and density of dentinal tubules are greater in the gingival margin than in the proximal wall. In addition, the water content is higher in the dentin at the gingival margin, making adhesion difficult. This is due not only to the amount of water already present within the demineralized matrix, but also to the fact that tubules contribute to contaminating the prepared surface with dentinal fluid. The cumulative effect of water increase leads to reduced adhesive infiltration and lower conversion of the adhesive to monomer/polymer (19).
Restorations of posterior proximal cavities with deep cervical margins below the CEJ (in the present case 1 mm) are more complex to treat than those above the CEJ. This procedure should be performed under rubber dam isolation, followed by matrix placement (20). However, margin control is a problem, as it requires careful evaluation of the interproximal contact point and the emergence profile.
Previous studies proposed specific matrix types for cervical margin elevation including circumferential and sectional matrices and stainless steel and transparent matrices (20-24) as well as matrices with curvatures that provide an adequate contact point and emergence profile and narrow subgingival fit (20-24).
Whatever the adhesion strategy used, the final common goal must be to obtain a compact and homogeneous hybrid layer, crucial for the stability of the adhesive bond over time (25). However, it must be considered that the structure of the hybrid layer changes according to the adhesive system used. The hybrid layer is a complex entity in which resinous monomers, collagen and hydroxyapatite, interact; therefore, aging phenomena can affect each component individually or simultaneously.
In *in vitro* studies, the stability of the hybrid layer is assessed by analyzing microleakage, using a silver nitrate tracer, which diffuses at the adhesive interface in areas not filled with resinous monomers (26). The longevity of the hybrid layer depends both on physical factors (occlusal forces, volume changes due to temperature change inside the oral cavity) and on chemical factors, which can come from the outside (acid agents present in saliva) or enzymes present within the dentin (27,28). The non-homogeneity of this layer leads to a progressive reduction of the bond strength due to the degradation caused by the disorganization of the collagen fibers and by the hydrolysis of the resin in the interfibrillar spaces (25). More specifically, hydrolysis is a process that breaks polymer chain bonds in the resin (29) mainly owing to water absorption, a phenomenon which is observed much more frequently in simplified adhesive systems (25,29). It should be emphasized that, regardless of the adhesive system used, poor polymerization leads to greater permeability and subsequent greater mobility of dentinal fluids (30). However, it should be noted that incomplete polymerization is more likely to occur with simplified than multi-pass adhesive systems, which are characterized by lower permeability (30). Besides hydrolysis of the resin, another fundamental factor in the degradation of the hybrid layer is the dentin's intrinsic collagenolytic activity (31-34). Studies carried out in recent years have shown that there are intrinsic enzymes capable of degrading collagen fibers even in the absence of bacterial enzymes (31). These enzymes belong to the family of MMPs (matrix metalloproteinases) and those most involved are MMP-2 and MMP-9 (33). It is believed that release and activation of these enzymes occur during the adhesion steps and are stimulated by the application of adhesive systems (30,34). The thinning and weakening of the collagen fiber network caused by the proteolytic action of these enzymes have been demonstrated by both *in vivo* and *in vitro* studies (30-34).
Therefore, microleakage of silver nitrate at the dentin-restoration interface is greater than at the enamel-restoration interface, most likely due to the chemical-physical characteristics of the substrate.

CONCLUSION

G2-Bond universal adhesive showed the best marginal sealing ability similar to Optibond FL both in the enamel and in the dentin. The adhesive interface in the enamel showed an extremely good seal while in the dentin, in all 3 groups tested, it always showed microleakage, regardless of the restorative material used.

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