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Factors Associated with Microleakage in Class II Resin Composite Restorations

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Clinical Relevance

Even though marginal gap size was not shown to be a direct predictor for the extent of microleakage in resin composite restorations, both material and placement technique appear to be important determinants in microleakage and, thus, probably in clinical outcomes.

SUMMARY

This *in vitro* study investigated the correlation between factors related to cavosurface marginal adaptation and microleakage in Class II cavities restored with a light- or chemical-activated resin composite. Standardized cavities were prepared in 40 molars that were randomly divided between both materials. Each of the groups was, in turn, divided, so that the restorations were placed by incremental and bulk techniques. The resultant four groups (n=10), each with material/technique variations, had their marginal gaps measured by environmental scanning electron microscopy at randomly selected facial and lingual points of the proximal box of each restoration. After sectioning the teeth, interfacial dye penetration was assessed by light stereomicroscopy according to an ordinal scale at the same locations as for the marginal gaps. In a general linear model with microleakage as a dependent variable, no correlation between marginal gap size and microleakage was found (p=0.802), although the interaction of the material and placement technique (p=0.028) and material alone (p=0.063) influenced microleakage. The model explained 63% of the variation in microleakage. It was concluded that, irrespective of the possible role of marginal gap in the occurrence of microleakage, the choice of material and placement technique are important determining factors in microleakage.

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INTRODUCTION

It is widely believed that microleakage associated with resin composite restorations can be directly linked to marginal integrity.[1] Marginal gaps are largely due to the shrinkage that resin composites undergo during polymerization.[2-4] Furthermore, since polymerization shrinkage is a direct function of the volume fraction of polymer matrix in composite, it occurs to a larger degree in microfine composites than in fine-particle composites or hybrids.[5] The rate and direction of polymerization contraction vectors in light- and chemical-activated materials also differ.[6]

While these parameters are inherent properties of a material that cannot be controlled by the clinician, restoration placement techniques can modify shrinkage stresses. Incremental placement of light-activated resin composites has been recommended to decrease the overall contraction by reducing the bulk of material cured at one time.[7-8] However, despite improvements in the formulation of new bonding agents with enhanced marginal adaptation and bond strengths, as well as clinical techniques designed to reduce the effects of shrinkage, a perfect marginal seal is still not achievable.[9]

The effects of microleakage include pulpal irritation, marginal discoloration and secondary caries.[10-12] These effects are due to the presence of bacteria, their nutrients or hydrogen ions, originating from plaque on the surface, leaking into the interfacial space.[1,13] Indeed, one of the main reasons for replacement of resin composite restorations is secondary caries, which accounts for 40-70% of dentists' stated reasons for doing so.[14-15] On the other hand, evidence is also growing that the relationship between marginal deficiency, microleakage and secondary caries may not be as clear-cut as is widely assumed.[16-17] A better understanding of this relationship would be useful for better consistency and accuracy in the diagnosis of secondary caries. Investigating any association between marginal gap and the factors that might influence its occurrence on the one hand, and microleakage on the other, would seem to be a useful area to explore this evidently complex clinical matter.

Investigations of marginal gaps have generally employed microscopy. A major limitation of optical microscopy is a lack of magnification and poor depth of field, while scanning electron microscopy (SEM) requires invasive sample preparation.[18] Environmental scanning electron microscopy (ESEM) allows for the examination of surfaces of hydrated, unfixed specimens in a non-destructive way, but with all the other advantages of conventional SEM.[19] Observations of microleakage, generally regarded as a functional measure of marginal quality, have been made using various methods. These methods include exposure to compressed air, bacteria and radioisotopes, but the easiest and most commonly-used method is dye penetration.[1]

This *in vitro* study investigated factors that may be associated with microleakage in Class II cavities in human molars restored with a light- or chemical-activated resin composite. Dye penetration and ESEM techniques were used to evaluate microleakage and marginal gaps. To control for the possible effect of placement technique, each material was placed both incrementally and in bulk.

METHODS AND MATERIALS

Selection and Preparation of Teeth

Forty freshly extracted human molars free of caries, restorations or other defects were selected. The teeth were cleaned of any tissue remnants on the roots and stored in physiologic saline with 0.05% sodium azide at 5°C for up to one month prior to use.

Mesio- or disto-occlusal Class II cavities were prepared to the following dimensions (±0.3 mm): 2.0 mm occlusal isthmus depth, following a conventional outline form; 3.0 mm facio-lingual proximal box width occlusally and 3.5 mm gingivally; 2.5 mm pulpal proximal box depth occlusally and 1.5 mm gingivally; 3.0-5.0 mm proximal box height, but always terminating at least 1.0 mm occlusal to the cemento-enamel junction so that all cavosurface margins were in enamel. All internal line and point angles were rounded. No bevels were placed at any of the cavosurface margins, but all margins were smoothed with white stones. All preparation and finishing procedures were carried out under copious water spray using a high speed handpiece.

Restoration Placement

The prepared teeth were randomly divided into two groups according to resin composite material (Table 1), cleaned with a pumice/water mixture in a rubber cup, rinsed in tap water and stored in distilled water until restored.

The teeth that were to be restored with light-activated material (Mat1) were totally etched (Ultra-Etch 35%, Ultradent, South Jordan, UT, USA) for 15 seconds

Table 1: *Distribution of Materials and Filling Techniques Within Total Sample (n=40 teeth)*

Material Group		Subgroup	Technique	Manufacturer
Mat1 (n=20)	Amelogen Universal	Tec1 (n=10)	Incremental	Ultradent, South Jordan, UT, USA
		Tec2 (n=10)	Bulk	
Mat2 (n=20)	Rapidfill	Tec1 (n=10)	Incremental	Mirage Dental Products, Kansas City, MI, USA
		Tec2 (n=10)	Bulk	

and rinsed with air/water spray. Excess surface water was removed by gently blowing with air and the dentin was left moist. Dentin primer (PermaQuik Primer, Ultradent), followed by dentin-enamel bonding resin (PermaQuik Bonding Resin, Ultradent), were applied, according to the manufacturer's instructions, and light-activated with a dedicated curing light (Max Curing Light, LD Caulk Co, Milford, DE, USA). Prior to placing the first restoration, the curing light was tested with a curing radiometer (Demetron/Kerr, Danbury, CT, USA) and the output intensity was maintained at 450 mW/cm^2 throughout the restorative procedures. A clear matrix (Premier Cure-Thru, ESPE-Premier, Norristown, PA, USA) was applied, and the resin composite material was placed randomly using one of two filling techniques: Tec1, incremental, oblique layering technique[8] (n=10); Tec2, bulk filling (n=10) (Table 1). For the first group (Mat1, Tec1), three successive increments of resin composite were placed, starting in the proximal box and were placed at a 45° angle at the facio-gingivo-proximal line angle. The composite was condensed, then cured for 40 seconds. Light-activation started at the facio-gingivo-proximal margin, followed by the linguo-proximal margin and terminated occlusally. Next, increments were placed and packed at the linguo-proximal box, and finally in the occlusal portion of the box and the isthmus. Light-activation of the second layer was done similar to the first layer, along with the occlusal addition from the occlusal direction. In the instance of Tec2, in order to minimize void formation, the material was syringed into the preparation, starting from the gingival floor, gradually depositing material as the syringe was withdrawn. When the preparation was slightly overfilled, the composite was lightly condensed, the excess removed and cured from three directions for 40 seconds each. Light-activation started at the facio-gingivo-proximal margin, proceeded to the linguo-gingivo-proximal margin and lastly from the occlusal direction.

The teeth to be restored with chemically-activated material (Mat2) were totally conditioned (WetBond Etch-Conditioner, Mirage Dental Systems, Kansas City, MI, USA), followed by an application of freshly-mixed adhesive resin (Mirage Dental Systems) according to the manufacturer's instructions. A matrix band, similar to that used for Mat1 teeth, was applied and, after mixing appropriate quantities of resin composite base and catalyst pastes for 20 seconds, the material was back-filled into a dose capsule, the plunger inserted and the capsule placed into the syringe. As for Mat1, the Mat2 group also had material placed on a random basis using 2 filling techniques: Tec1, placed incrementally, using the same layering technique as Mat1, Tec1 (n=10); Tec2, bulk filling (n=10) (Table 1). For the Mat2, Tec1 group, the syringe containing mixed resin composite was deposited into the cavity incrementally and condensed, following the sequence as described for Mat1, Tec1. For the second and third increments, fresh quantities of composite were mixed, loaded into the dose capsule and deposited into the cavity. When the cavity was filled just beyond the level of the occlusal surfaces, the excess material was removed. During bulk filling for Mat2, Tec2, void formation was minimized in the same way as for Mat1, Tec2.

All restorations were finished wet immediately after resin placement following the manufacturer's recommendations (Sof-Lex, 3M Dental Products Division, St Paul, MN, USA). The teeth were stored in distilled water for 24 hours at 35°C before thermocycling, which comprised 500 cycles (20 seconds in a 55°C water bath, followed by five seconds in a 5°C water bath, with a dwell time of 30 seconds).

Marginal Gap Measurement

Measurements of marginal gap widths were made at a randomly selected point on each of the facio-proximal and linguo-proximal cavosurface margins of each restoration. An ESEM (ElectroScan Corporation, Wilmington, MA, USA) was used according to a method previously described.[20-21] The specimens were mounted on a standard half-inch pin-type aluminum stub coated with carbon paint. The stubs were placed on the ESEM specimen stage. Due to the precise location fixing capability of the ESEM on the x, y and z axes, it was possible to orient the interfacial plane being observed so that a gap width could be obtained using the image analyzing software. ESEM analysis was performed at an accelerating voltage of 25 kV, and water vapor (the imaging gas in the sample chamber) was held at a pressure of 4.6-6.7 torr. The stereo-zoom at original magnification 350x was used for all measurements, and samples were randomly measured to eliminate operator bias.

Microleakage Evaluation

Following ESEM measurements, the apices of the teeth were sealed with a chemically-cured resin composite (Rapidfill, Mirage Dental Systems). Two layers of nail varnish were successively applied (allowing the first layer to dry before applying the second) to the entire surface of the teeth to within approximately 1 mm of the cavosurface margin of the restoration. The specimens were immersed in 5% methylene blue at 37°C for 24 hours, rinsed in tap water and embedded in epoxy resin (Epofix, EMS, Fort Washington, PA, USA). They were then sectioned mesio-distally and approximately horizontally using a low-speed diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA) so as to traverse the facial and lingual margins of the proximal box at the points at which the marginal gaps had been measured.

All specimens were observed under a Leica 1660 light stereomicroscope (Leica Instruments GmbH, Nussloch, Germany) at 60x original magnification. Maximum penetration of the dye was graded according to the following ordinal scale: 0=no dye penetration; 1=dye penetration in enamel only; 2=dye penetration to dentin-enamel junction (DEJ); 3=dye penetration beyond DEJ; 4=dye penetration >2 mm into dentin.

Data Analysis

Mean (± SD) gap measurements and frequency distributions of the microleakage scores were calculated for each filling technique within each material, using the tooth (n=40) as the unit of analysis. Identification of factors that could possibly have an influence on microleakage, namely, marginal gap size, the material and filling technique used and any interactions thereof, were first sought through graphical display of any correlations. Finally, regression analysis was performed, in which a general linear model was fitted with microleakage as the dependent variable. Analyses were performed using a statistical program (SYSTAT 8.0, Evanston, IL, USA), and the level of significance was set at $p<0.05$.

RESULTS

The difference in mean gap size between materials was statistically significant (Mat1: 3.56 µm, SD 1.53; Mat2: 2.13 µm, SD 1.13) ($p<0.05$). Figure 1 displays the frequency distributions of microleakage scores for the groups. Mean microleakage observed in Mat1 (2.73, SD 0.55) was also greater than that observed in Mat2 (1.18, SD 0.86) ($p<0.05$).

The correlation between marginal gap size and microleakage, ignoring the effects of material and technique, is shown graphically in Figure 2, while inclusion of the effects of the different materials and techniques produced the regression lines shown in Figure 3. The final general linear model is summarized in Table 2, confirming the significant associations with microleakage to be the interaction of material and filling technique and material alone. The interaction of material and marginal gap as an influential factor in microleakage was mildly apparent, while that of the marginal gap alone was not. The model explained 63% of the variation in microleakage.

DISCUSSION

A limitation of the micro-morphological examination of restoration margins by a non-destructive method such as ESEM is that it can only determine the quality of the interface at its external aspect, namely the cavosurface margin.[22] The presence of interfacial spaces extending along the axial wall is usually assessed by microleakage studies. Whether the former variable (marginal gaps) can be predictive of the second (interfacial spaces)

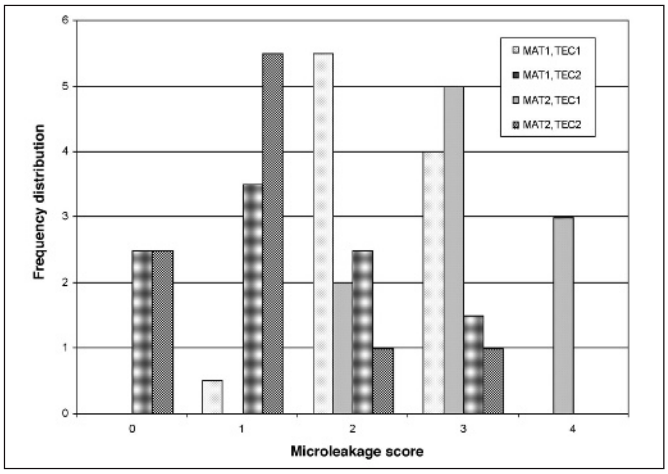

Figure 1: *Frequency distribution of dye penetration scores for the groups (MAT,TEC legend indicates variations of materials and filling techniques: MAT1,TEC1 light-activated, incremental fill [n=10], MAT1,TEC2 light-activated, bulk fill [n=10], MAT2,TEC1 chemically-activated, incremental fill [n=10], MAT2,TEC2 chemically-activated, bulk fill [n=10]).*

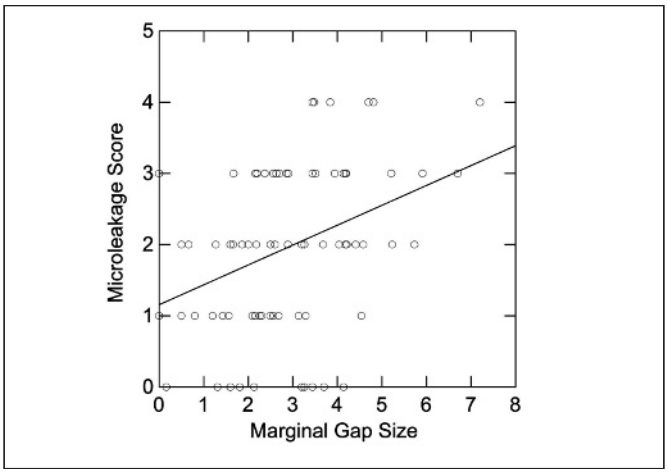

Figure 2: *Correlation (regression line) between marginal gap size (µm) and microleakage, ignoring the effects of material and technique (n=40).*

Table 2: *General Linear Model for Microleakage (R^2=0.634)*

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Material	1.742	1	1.742	3.681	0.063
Technique	0.472	1	0.472	0.998	0.325
Marginal gap	0.030	1	0.030	0.064	0.802
Material*Gap	0.486	1	0.486	1.028	0.318
Technique*Material	2.496	1	2.496	5.276	0.028
Error	16.086	34	0.473		

*Note: * = interaction factors*

variable to occur remains an important research question. Attempting to clarify the strength of the claim for this relationship was the main thrust of this investigation.

Finding greater microleakage in light-activated (Mat1) compared to chemically-activated (Mat2) restorations was seemingly in line with the pattern of mean cavosurface marginal gap quantifications observed. While this appears to concur with the accepted view that both of these acknowledged measures of marginal characterization are related,[1,23] the picture is more complex, as shown by the results of subsequent regression analysis. For this purpose, the unit of analysis chosen was the tooth, with facial and lingual sites considered as a mean. Furthermore, the ordinal scale used to score microleakage was considered sufficiently quantitative in its meaning to be quasi-interval, and thus, permits the application of a more powerful parametric rather than a non-parametric test in the analysis of data.[24]

When a resin composite is used to restore a tooth, any resultant marginal gap reflects an interaction between polymerization shrinkage, bond strength and material flow during the setting period.[25] Of these contributory factors, setting shrinkage-strain has been shown to have the greatest effect on marginal adaptation,[23,26] with light-activated materials more adversely affected in this regard than chemical-activated materials.[9] This is due to chemical-activated resin composites polymerizing more slowly, having a greater capacity to flow during their longer gel stage and, thus, generating lower stresses on formation of the adhesive bond.[26] These results would seem to be in agreement with this pattern.

Because the design of this exploratory study sought to identify possible explanatory factors for microleakage in Class II resin composite restorations, not only the effects of the material, but also the placement technique were included. It has been reported that mean marginal gap sizes are independent of filling technique.[21,27] In this study, regression analysis failed to show correlations between the independent variables of technique, marginal gap size or their interactions with microleakage. In fact, the only influences of significance were interaction of the material and filling technique (Table 2, Figure 3).

This study's main finding—that of no significant correlation between cavosurface marginal dimension and microleakage—is at variance with the concept that the two parameters are invariably, and perhaps even proportionately linked. In another recent study, evidence of a consistent correlation between marginal gap size and microleakage in Class II composite restorations was also not found.[28] On the other hand, a study of Class V composite restorations reported similar rankings in enamel for gap size and microleakage assessed

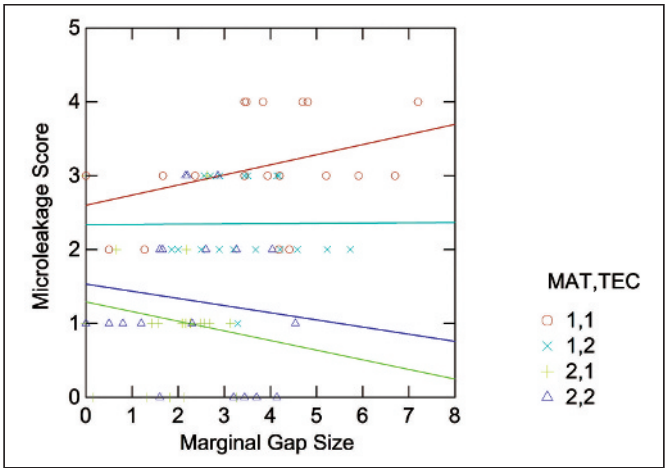

Figure 3: *Correlations (regression lines) between marginal gap size (μm) and microleakage, allowing for the effects of material and technique (MAT,TEC legend indicates variations of materials and filling techniques: MAT1,TEC1 light-activated, incremental fill [n=10], MAT1,TEC2 light-activated, bulk fill [n=10], MAT2,TEC1 chemically-activated, incremental fill [n=10], MAT2,TEC2 chemically-activated, bulk fill [n=10]).*

by SEM and dye penetration, respectively, but found more inconsistent rankings in dentin.[29] This might partly be explained by the wider biological variation of dentin, leading to a less reliable adhesive bond to resist the effects of polymerization shrinkage and, thus, gap formation.[22] It might also be due to internal void formation.[30] In this regard, whereas one study reported no correlation between the extent of microleakage and the presence of internal voids,[31] another study has more recently found a positive association between them.[4] It would seem that, when comparing external and internal adaptations of tooth-restoration interfaces, there is no clear correlation between them,[32] which raises a question about the role of cavosurface marginal integrity *per se* in influencing microleakage. At the clinical level, what this means in terms of the perceived risk of secondary caries, is equally unclear.[17]

On the other hand, it is the prevailing consensus that marginal integrity is an important means of preventing secondary caries, since it can control microleakage.[33] Thus, the quest for a perfect seal between the restoration and cavity walls remains a goal of many researchers.[1] Marginal adaptation and microleakage might be complementary in a comprehensive characterization of the adhesive interface, especially the integrity of the bond between the adhesive system and substrates. However, the absence of a correlation between these accepted measures of marginal quality in this study points to the possibility of other related factors being influential in the process. In this regard, the role of material and the placement technique of restorations, as well as the material itself, appears to be important. Further research is needed to clarify some of the complexities of the mechanisms by which the latter factors have an impact on microleakage.

CONCLUSIONS

Under the conditions of this study, no correlation between cavosurface marginal gap size and degree of microleakage was found. On the other hand, the interactive effect of restorative material and filling technique, and the effect of material alone, were associated with microleakage. This suggests that light- and chemical-activated resin composite restorations placed incrementally or in bulk have the potential to influence microleakage. Regardless of whether marginal gap can be directly implicated in the occurrence of microleakage, the choice of material and placement technique would seem to be more important determining factors in any relationship between the 2 characteristics.

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