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Composite Replacement of Amalgam Restoration Versus Freshly Cut Dentin: An *In Vitro* Microleakage Comparison

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Clinical Relevance

Bonding to dentin under replaced amalgam restorations may be as effective as bonding to freshly cut dentin.

SUMMARY

Objective: The aim of this study was to evaluate the microleakage of the composite restorations when bonded to tooth structure previously restored with amalgam material compared with that of freshly cut dentin.

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Methods and Materials: Thirty intact, extracted intact human molars were mounted in autopolymerizing acrylic resin. Class II box preparations were prepared on the occluso-proximal surfaces of each tooth (4-mm bucco-lingual width and 2-mm mesio-distal depth) with the gingival cavosurface margin 1 mm above the CEJ. Each cavity was then restored using high copper amalgam restoration (Disperalloy, Dentsply) and then thermocycled for 10,000 thermal cycles. Twenty-five of the amalgam restorations were then carefully removed and replaced with Filtek Supreme Ultra Universal (3M ESPE); the remaining five were used for scanning electron microscopy and energy dispersive x-ray spectroscopy analysis. A preparation of the same dimensions was performed on the opposite surface of the tooth and restored with composite resin and thermocycled for 5000 thermal cycles. Twenty samples were randomly selected for dye penetration testing using silver nitrate staining to detect the microleakage. The specimens were analyzed with a stereomicroscope at a magnification of 20×. All of the measurements were done in micrometers; two readings were taken

for each cavity at the occlusal and proximal margins. Two measurements were taken using a 0-3 scale and the percentage measurements.

Results: Corrosion products were not detected in either group (fresh cut dentin and teeth previously restored with amalgam). No statistically significant difference was found between the microleakage of the two groups using a 0-3 scale at the occlusal margins (McNemar test, p=0.727) or proximal margins (Wilcoxon signed-rank test, p=0.174). No significance difference was found between the two groups using the percentage measurements and a Wilcoxon signed-rank test at either the occlusal (p=0.675) or proximal (p=0.513) margins. However, marginal microleakage was statistically significant between the proximal and occlusal margins (p<0.001).

Conclusion: Within the limitations of this *in vitro* study, no significant difference was found between the microleakage of nondiscolored dentin in teeth that were previously restored with amalgam compared with freshly cut dentin. However, marginal microleakage in the proximal surface was higher than that in the occlusal surface.

INTRODUCTION

The use of composite resin material has increased and has become the first choice for most carious lesions. This change can be attributed to several factors.[1] Some of the main factors are that amalgam restorations do not adhere to the cavity wall chemically, and there are critical differences in the coefficient of thermal expansion of amalgam and tooth structure. Amalgams allow the transport of fluids, ions, molecules, and possibly bacteria and their toxins more readily.[2,3] These problems are more obvious when poor operative technique is used. Moreover, mercury used in amalgams has raised concerns about its biological toxicity and environmental hazards. People are exposed to mercury and other metals via vapor and corrosion products in swallowed saliva and by direct absorption through the oral mucosa.[4-6] However, appearance, in addition to the controversy about mercury toxicity, is the public's displeasure with amalgam. Additionally, amalgam has other disadvantages, in that corrosion can cause increased porosity and reduced marginal integrity and strength, as well as the release of metallic products into the oral cavity.[4] The literature supports a lack of evidence that amalgam and its mercury content can be harmful to humans, with the only exception being specific metal allergies.[7]

The use of tooth-colored restorative material rather than amalgam restorations requires the meticulous use of an adhesive technique.[8] This is because dentin is more hydrophilic in nature compared with enamel and therefore is more difficult to deal with in terms of bonding to adhesive resin. However, enamel is known to have reliable bond strength when bonded to resin-based composites, because its main components are inorganic, whereas dentin has a different composition and structure.[9,10]

Although various generations of bonding agents have been developed to decrease polymerization shrinkage, microleakage remains a significant problem[11] and a major factor that affects the longevity of composite restorations.[12] This is a primary cause of marginal discoloration, secondary caries, pulpal inflammation, postoperative sensitivity, and restoration replacement.[13] Microleakage measurements usually provide an assessment of the sealing ability of adhesive materials, which is clinically relevant.[14]

According to Abo and others,[15] an *in vitro* microleakage test combined with thermocycling is a useful method to assess sealing performance. In their study, they also demonstrated that the use of a large number of thermal cycles could simulate the conditions in the oral environment. In a review performed by Heintze[16] on the evaluation of in vitro sealing ability, microleakage testing was preferred over other methods because of its ease and simplicity. The second most frequent method, the quantitative marginal analysis of replicas using scanning electron microscopy (SEM) is used less often because it is more time consuming and complex[16]

In many clinical situations, adhesive resin is used to bond to sclerotic dentin or dentin discolored by corrosion products from the amalgam restorations that have penetrated the dentinal tubules. Several studies have shown that the bond strength was lower than or similar to that of unaffected dentin.[17-19] If a poor technique is used, or contamination occurs during amalgam placement, it may elicit dentin staining, as can the presence of recurrent caries. Even in the late 19th and early 20th centuries, it was suggested that dentin staining could be caused by the deposition of metallic sulfides and the penetration of silver and mercury ions from the overlying amalgam.[19-25] Scholtanus and others[2] concluded that the discoloration of dentin beneath amalgam was an indicator of the presence of amalgam constituents, the effect of which is unknown in adhesive restor-

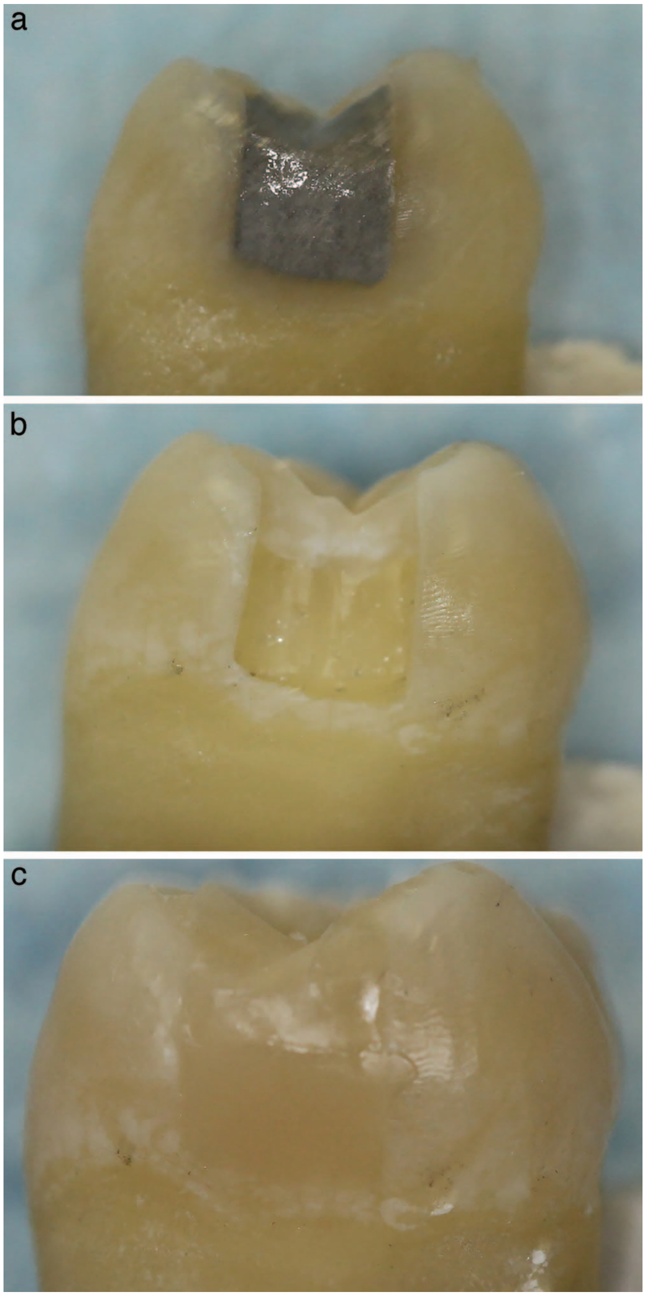

Figure 1a. *Restoration process a. amalgam restoration placement.*
Figure 1b. *Cavity walls after removal of the amalgam restoration.*
Figure 1c. *Composite resin placement.*

ative procedures. Harnirattisai and others[25] showed that bond strength to dark dentin after amalgam removal is lower than that to normal dentin. Data are lacking in the literature about microleakage of composite resins after amalgam removal.

Therefore, the purpose of this *in vitro* study was to evaluate the microleakage of composite restorations when bonded to a cavity previously restored with amalgam material compared with that of freshly cut dentin. The hypothesis was that there would be more microleakage in a composite restoration when bonded to a cavity previously restored with amalgam material compared with that of freshly cut dentin.

METHODS AND MATERIALS

Sample Selection and Preparation

Thirty intact, caries-free, extracted human molar teeth were collected from the general collection jar in the Oral Surgery Department at Tufts University School of Dental Medicine. Twenty teeth were used for the microleakage test and 10 for the SEM and energy dispersive x-ray (EDX) analysis.

The teeth were placed in autopolymerizing acrylic resin (Technovit, Heraeus Kulzer, Wehrheim, Germany) using a plastic mounting template (Ultradent, South Jordan, UT, USA) with the experimental surface of the teeth exposed. The teeth were stored in distilled water at room temperature at all times.

Fabrication of the Restoration

Standardized class II box preparations were prepared on the occluso-proximal surfaces of each tooth (4-mm bucco-lingual width and 2-mm mesio-distal depth) with the gingival cavosurface margin 1 mm above the cemento-enamel junction (CEJ) and the cavosurface margin a butt joint,[26] using #245 carbide burs (SS White, Lakewood, NJ, USA) in an air/water-cooled high-speed turbine. A new bur was used after every five preparations.[27]

A Tofflemire matrix band was placed, and the restoration procedure began with the application of two layers of varnish (Copalite, Cooley & Cooley LTD, Houston, TX, USA) according to the manufacturer's instructions. Each cavity was then restored using dental amalgam (Dispersalloy, Dentsply, Milford, DE, USA) (Figure 1a). All teeth were thermocycled for 10,000 thermal cycles between water baths at 5°C and 55°C with a 30-second dwell time. This procedure aged the material to simulate a year of clinical performance and to generate amalgam corrosion products.[28]

Next, the amalgam restoration was removed carefully using #245 carbide burs (SS White) in an air/water-cooled, high-speed turbine (Figure 1b). To prevent encroachment on the dentin, the final layer of amalgam was removed with an explorer.[29] The amalgam was removed from all the samples except five, which were used for the SEM and EDX analysis.

A cavity of the same dimensions was prepared on the other side of the tooth using #245 carbide burs (SS White) in an air/water-cooled high-speed tur-

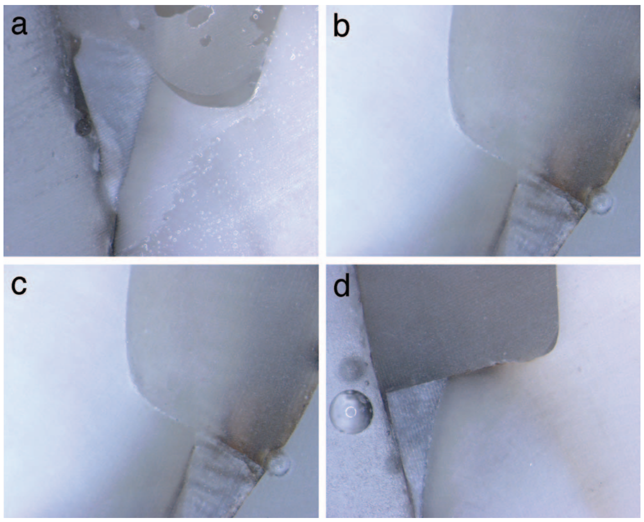

Figure 2a. *Microleakage at the proximal margin a. Score 0.*
Figure 2b. *Score 1.*
Figure 2c. *Score 2.*
Figure 2d. *Score 3.*

bine. A Tofflemire matrix band was placed to ensure that the light curing of the restoration occurred only from the occlusal side of the restoration. Both cavities were restored with composite material using a total-etch adhesive system.

The composite restoration began by etching the enamel with 35% phosphoric acid (Ultra-Etch, Ultradent) for 20 seconds and dentin for 15 seconds. The teeth were rinsed with water for 10 seconds and blot dried with cotton pellets to achieve a moist dentin surface and then were bonded with total-etch adhesive (ExciTE F DSC, Ivoclar Vivadent, Amherst, NY, USA) that was applied to the enamel and dentin surfaces, then agitated for 10 seconds and light cured according to the manufacturer's recommendations.

The teeth were restored with composite resin material (Filtek Supreme Ultra Universal, 3M ESPE, St. Paul, MN, USA) via the Liebenberg technique.[30] A blue light emitting diode (LED) was used at 800 mW/cm^2, followed by finishing and polishing (Figure 1c). The light source was monitored every five curing cycles using a radiometer (Demetron L.E.D, Kerr Corp., Orange, CA, USA) to ensure that the light intensity remained stable.[31] All the teeth were thermocycled for 5000 thermal cycles between water baths at 5°C and 55°C with a 30-second dwell time, which served to age the material to simulate clinical performance.

Microleakage Test Preparation

All of the samples were coated with two layers of nail polish (Vinyl shine nail polish, Rimmel London, London, UK), except for a 2.0-mm rim around the restoration to allow the leakage-tracing agent to contact the margins of the restoration. Thereafter, the teeth were immersed in a solution of 50 wt% ammoniacal silver nitrate (pH=9.5) (Fisher Scientific, Fair Lawn, NJ, USAQ) for 24 hours, followed by eight hours in a photo-developing solution (Eastman Kodak Co., Rochester, NY, USA).[32]

The specimens were washed under running water for one minute. The nail polish was removed carefully with a #15 scalpel, after which the specimen was embedded in epoxy material (Epoxicure resin, Buehler Ltd., Buehler, IL, USA). Each tooth was sectioned mesiodistally with a diamond saw, and the blocks were positioned in a precision cutting machine (1000 Isomet, Buehler Ltd.).

The specimens were analyzed with a stereomicroscope (Olympus America, Inc., Center Valley, PA, USA) at a magnification of 20×. All of the measurements were made in micrometers; two readings were taken for each cavity (occlusal and cervical = four readings per tooth).[33] The specimens were scored according to the following degree of dye penetration (Figures 2 and 3)—occlusal score: 0, no dye penetration; 1, dye penetration into enamel; 2, dye penetration beyond the dentinoenamel junction; 3, dye penetration into the pulpal wall; proximal score: 0, no dye penetration; 1, dye penetration into enamel; 2, dye penetration up to half extension of cervical wall; 3, dye penetration into more than half or complete extension of the cervical wall.[34]

The dye penetrations were also recorded in percentage measurements using the following formula[33]: leakage number = (distance evidenced for dye/overall distance for margin) × 100.

SEM and EDX

All of the samples were embedded in epoxy resin material (Epoxicure resin, Buehler Ltd.). Each sample was sectioned mesiodistally with a diamond saw (1000 Isomet, Buehler Ltd.). Each section was mounted on an Hitachi table (Aluminum mount, Electron Microscopy Science, Hatfield, PA, USA) with conductive adhesive (Graphite conductive adhesive, Electron Microscopy Science). The samples were coated with a 4.5-μm platinum layer using a coating machine (Sputter coater 208hr, Cressington, Watford, UK). Each section was analyzed with a scanning electron microscope (Hitachi S-48000, Hitachi, Tokyo, Japan) and EDX to analyze the metal penetration in the dentinal tubules below the interface between the restoration and the dentin, as

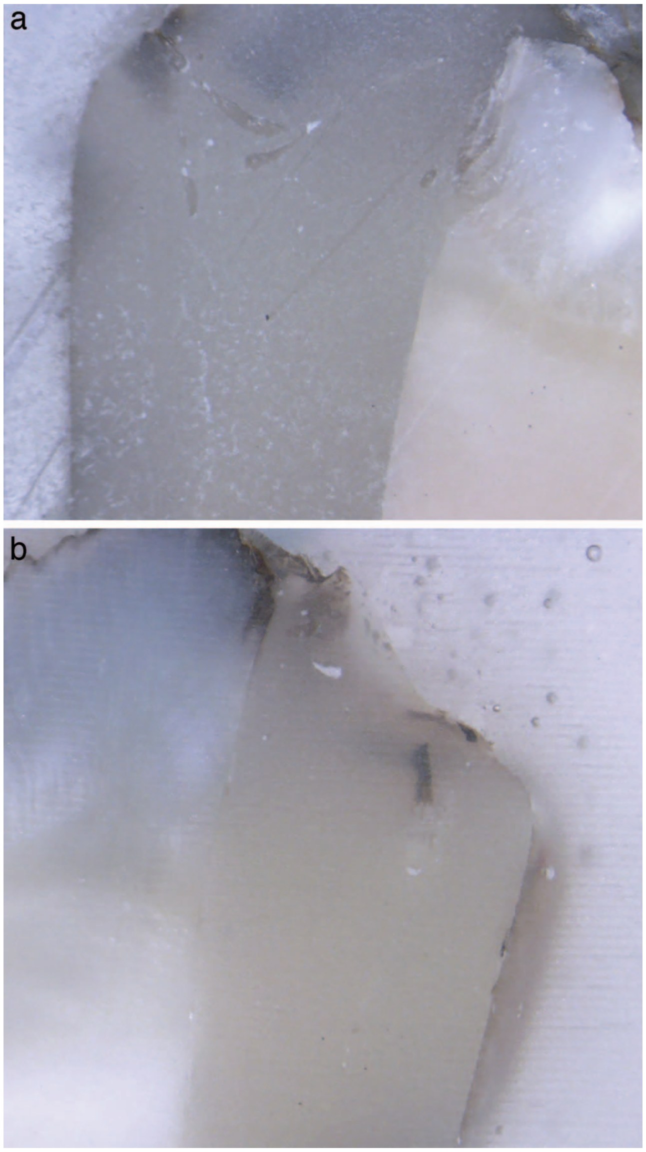

Figure 3a. *Microleakage at the occlusal margin a.Score 0.*
Figure 3b. *Score 1.*

well as at the interface. All analyses were conducted using EDAX Genesis software (EDAX Inc., Mahwah, NJ, USA) operated at 20 Kv.

Statistical Analysis

A power calculation was performed using nQuery Advisor (Version 7.0). Assuming an 84% chance for the group with nonpreviously restored teeth to have lower microleakage than the group with previously restored teeth,[35] a sample size of $n = 20$ teeth was adequate to obtain a type I error rate of 5% and a power greater than 99%.

For the 0-3 scale of microleakage at the cervical surface, counts and percentages were calculated, and statistical significance was assessed via the Wilcoxon signed-rank test. For the occlusal score, counts and percentages were calculated, and statistical significance was assessed via the McNemar test. For the percentage scale of microleakage, median and interquartile ranges were calculated, and statistical significance was assessed via the Wilcoxon signed-rank test, because the assumption of normally distributed data was violated; p-values less than 0.05 were considered statistically significant. (IBM SPSS Statistics 21, IBM Corp., Armonk, NY,USA) was used in the analyses.

Table 1: *Counts (Percentages) for 0-3 Microleakage Scale at Occlusal Margin*

Tested groups at occlusal margins	0	1	*p* Value
Previous amalgam	12 (60%)	8 (40%)	0.727
Fresh-cut dentin	10 (50%)	10 (50%)	

RESULTS

Microleakage Test

Dye penetration and consequently microleakage varied between the occlusal and proximal surfaces.

Occlusal Marginal Microleakage—The results of microleakage on a 0-3 scale are presented in Table 1. For the group of teeth that were previously restored with amalgam, 12 teeth (60%) had a score of 0, and eight teeth (40%) had a score of 1; for the fresh-cut dentin group, 10 teeth (50%) had a score of 0, and 10 teeth (50%) had a score of 1. The McNemar test revealed no significant difference in microleakage for the two groups at the occlusal surface (p=0.727).

Percentage measurement data are presented in Table 2. The median value for teeth that were previously restored with amalgam was 0%, whereas it was 13%; for the fresh-cut dentin group. The minimum percentage of microleakage for the amalgam group was 0%, with a maximum value of 16%. The minimum microleakage for the fresh-cut dentin was also 0%; the maximum value, however, was 33%. No significant difference was found between the groups (p=0.675).

Proximal Marginal Microleakage—The results of the microleakage on a 0-3 scale are presented in Table 3. For the teeth that were previously restored with amalgam, 18 teeth (90%) had a microleakage score of 2 or 3. For the fresh-cut dentin group, 13 teeth (65%) had a microleakage score of 2 or 3. The overall distribution of the microleakage scores did

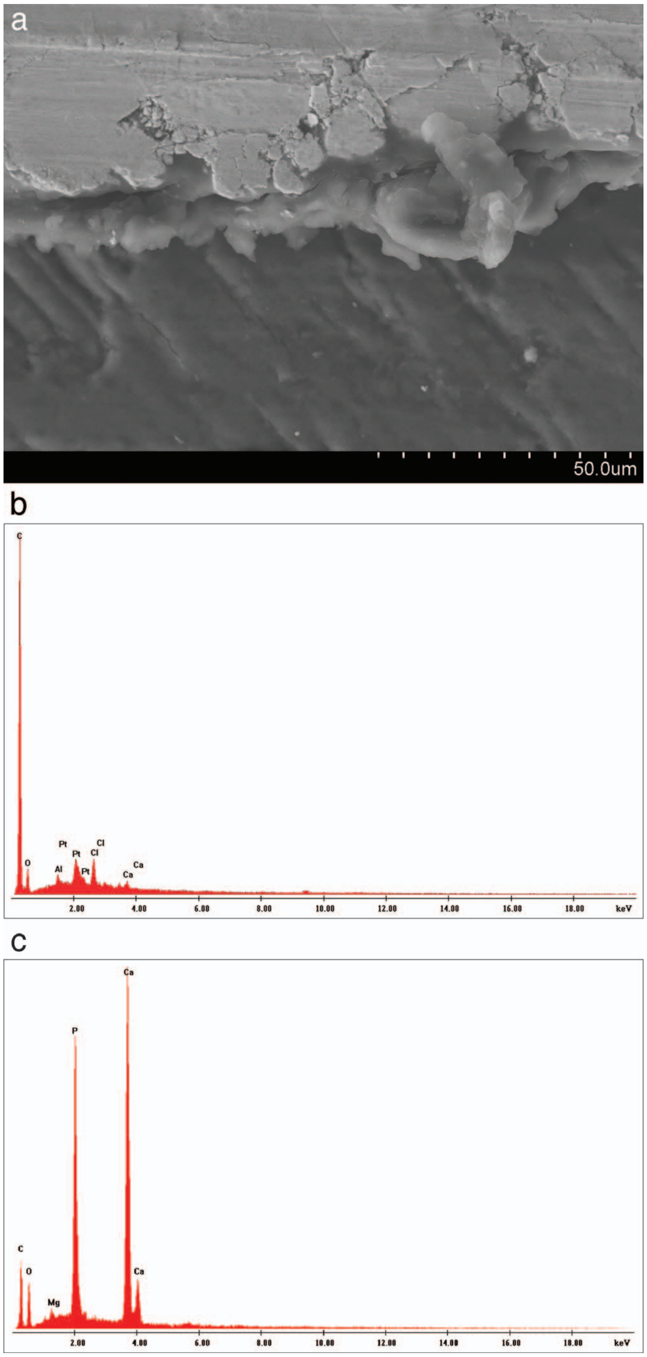

Figure 4a. *SEM of the dentin-amalgam interface showing the varnish layer.*
Figure 4b. *EDX analysis for varnish layer.*
Figure 4c. *EDX analysis for the dentin below the amalgam restoration.*

not differ significantly between the two groups (p=0.174).

Percentage measurement results are presented in Table 4. The median value for teeth that were previously restored with amalgam was 78% and for the fresh cut dentin was 67%. The minimum percentage of the microleakage for the amalgam group was 39%, with a maximum value of 100%. Although the maximum microleakage for the fresh-cut dentin group was also 100%, the minimum value was 0%. No significant difference was found between the groups (p=0.513). However, a statistically significant difference was found between the occlusal and proximal margins (p<0.001).

SEM and EDX

The results of the SEM and EDX are presented in graphs and elemental analysis charts.

Teeth With Amalgam Restorations (Figure 4a)—Several areas were analyzed (amalgam restoration, varnish layer, dentin surface, and the dentinal tubules beneath the restoration). The varnish layer revealed that chlorine was associated with the chloroform in the varnish composition (Figure 4b) and platinum metal from the coating. No metal was detected in the dentinal tubules below the amalgam restoration that was thermocycled for 10,000 or 15,000 cycles (Figure 4c).

Teeth That Were Previously Restored With Amalgam (Figure 5a)—No metal elements from the amalgam corrosion products were found in the dentinal tubules and the dentin surface beneath the composite restoration. However, silicon was detected and was found to be associated with the dentin adhesive (Figure 5b).

Teeth With Fresh-cut Dentin (Figure 6a)—Silicon was also detected to be associated with the dentin adhesive. However, there were differences between the amount of silicon in the hybrid layer and the dentin beneath the restoration (Figure 6b).

DISCUSSION

In this study, dye penetration testing was chosen because it is the most widely used method of evaluation and is the gold standard. Dye penetration also represents the most reliable quantitative mea-

Table 2: *Descriptive Statistics for the Percentage Microleakage Score at the Occlusal Margin*

Tested groups at occlusal margins	*N*	Median	Interquartile range	Minimum	Maximum	*p* Value
Previous amalgam	20	0	8	0	16	0.675
Fresh-cut dentin	20	13	7	0	33	

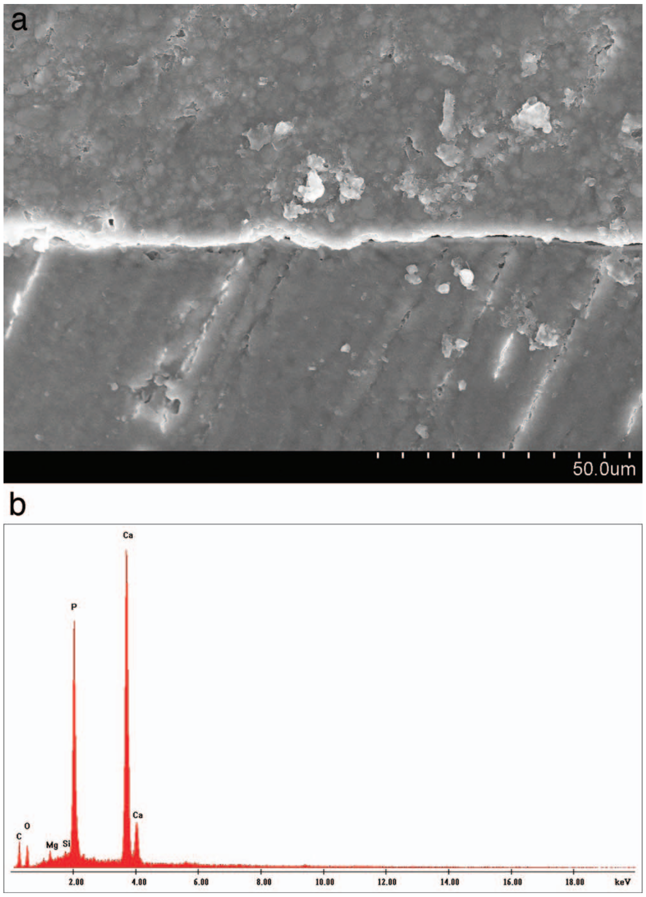

Figure 5a. *SEM of the dentin-composite interface that was previously restored with amalgam.*
Figure 5b. *EDX analysis for the dentin below composite restoration.*

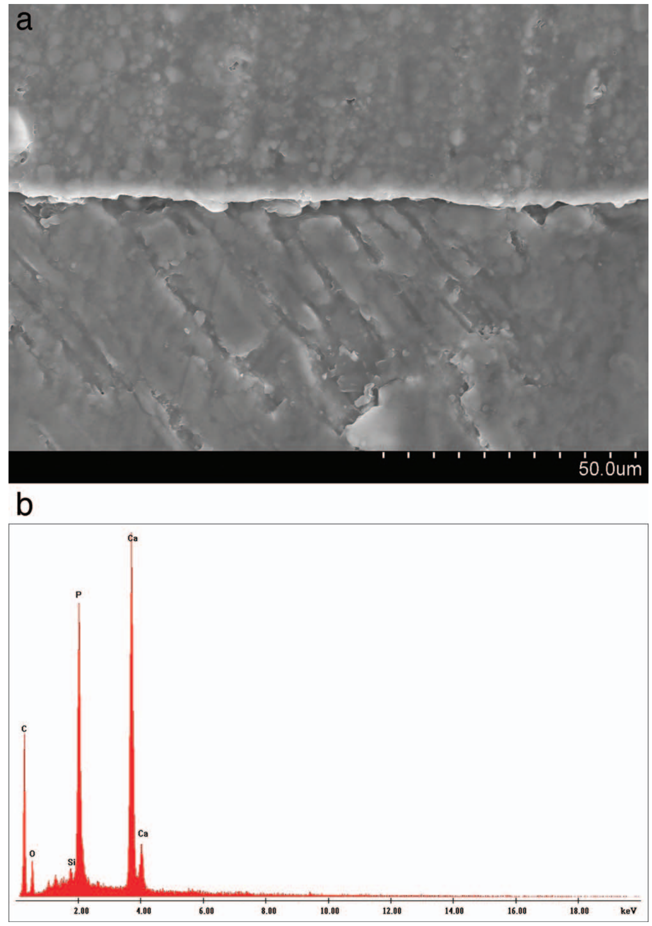

Figure 6a. *SEM of the dentin-composite interface for fresh-cut dentin.*
Figure 6b. *EDX analysis for the dentin below the composite restoration.*

surement of microleakage. One of the goals of an ideal restoration is to prevent microleakage, as this is an important aspect of the longevity of restorations. Moreover, it represents the passage of bacteria at the tooth restoration interface, which may cause recurrent caries or pulpal irritation and subsequent pulpal inflammation.[36]

Two measurements were used to evaluate the dye penetration at the proximal and occlusal margins to confirm whether or not different measuring techniques might affect the results. Hypothesis tests based on the two measurements led to the same conclusion. All teeth previously restored with amalgam restorations had 90% microleakage at the proximal margin with scores of 2 or 3. In contrast, the other group showed 65% microleakage with the same scores.

This may be due to the effect of the cavity preparation and application of a cavity varnish layer. The smear layer and subsequent contaminants could block the dentinal tubules from forming a smear plug. Contaminated dentin could be exposed to a variety of ions and molecules originating from amalgam and oral fluids. Further, differences in the coefficient of thermal expansion between amalgam and tooth structure can result in intermittent opening and closing of the gap, thus

Table 3: *Counts (Percentages) at the Proximal Margin*

Tested groups at proximal margins	**0**	**1**	**2**	**3**	***p* Value**
Previous amalgam	0 (0%)	2 (10%)	12 (60%)	6 (30%)	0.174
Fresh cut dentin	2 (10%)	5 (25%)	8 (40%)	5 (25%)	

Table 4: *Descriptive Statistics for the Percentage Microleakage Score at the Proximal Margin*

Tested groups at proximal margins	*N*	Median	Interqartile range	Minimum	Maximum	*p* Value
Previous amalgam	20	78	38	39	100	0.513
Fresh-cut dentin	20	67	52	0	100	

creating inward and outward transport of fluids along the amalgam-tooth interface. Dentin is exposed to saliva and products of bacterial metabolism more readily,[2] and these factors elucidate the difference between the dentin of teeth that were previously restored with amalgam compared to fresh-cut dentin.

The findings of this study contrasted with those of Ghavamnasiri and others,[29] who found a difference in microleakage between teeth that were previously restored with amalgam vs with freshly cut dentin. This difference may be related to varied composition or solutions, as Ghavamnasiri and others used chloroform-free varnish. Other differences that may have had an influence on the results include dye tracer and storage methods. According to Heintze and others,[37] different tracers produce different results, especially at the dentin margin.

The EDX analysis in this study revealed the presence of calcium and phosphorus, elements that are correlated with the composition of dentin.[38] The findings of this study are consistent with those of Wei and Ingram, Kurosaki and Fusayama, and Halse and others, who concluded that metal did not penetrate into nondiscolored dentin. They found that penetration of metals, such as Zn and Sn, occurred exclusively in the dark, discolored dentin.[39-41] The review of the literature by Scholtanus and others[2] confirmed that penetration of metals from amalgam was observed only in discolored and demineralized dentin.[2,25,40-43]

However, these results conflict with that of the research of Ghavamnasiri and others,[29] as tin, silver, mercury, and copper were found in the dentin adjacent to the composite restoration after the amalgam replacement. Moreover, high copper amalgam was used, and it has been reported that no mercury is released by the corrosion process. Corrosion of high copper amalgam with in vitro experiments revealed Ca-Sn-P-Cl complexes and crystalline products containing Sn at the amalgam-tooth interface. No Cu was found as Cu complexes were assumed to be leached out into the liquid environment.[2,29]

The findings of this study conflict with those of the study of Grossman and Matejka,[38] as several metal elements were found in the dentin and enamel after the placement of the amalgam restorations. However, a conventional amalgam was used, and the samples were stored in 1% NaCl solution for one year, which may have affected the corrosion process or products. Moreover, Soremark and others[44] also found an increase in the concentrations of mercury and silver in the dentin and enamel after the amalgam restoration, as well as a moderate increase in tin and zinc. In this particular study, the researchers did not specify the amalgam type, which has a significant effect on the corrosion products.

The EDX analysis of the dentin below the composite restorations also revealed differing amounts of silicon, which related to the application of the adhesive. This finding contrasts with that of Harnirattisai and others,[25] who used Single Bond (3M ESPE) and Clearfil SE Bond (Kuraray, New York, NY, USA) and did not find elements related to the adhesive in the adjacent dentin. Our findings are consistent with those of Ghavamnasiri and others,[29] who reported the presence of large amounts of tin, barium, and silicon; the concentrations of both tin and barium were related to the opaque metal included in the adhesive.

Although there was no statistically significant difference between the two groups, and metal did not penetrate the nondiscolored dentin, the teeth previously restored with amalgam restorations showed more microleakage (39% to 100%) compared with the freshly cut dentin that had (0% to 100%). These findings suggest that cavity preparation should be extended beyond the removal of an amalgam restoration. Ghavamnasiri and others[29] also recommended the removal of approximately 0.5 mm of nondiscolored dentin to improve the gingival microleakage and achieve the same level as that obtained with the fresh dentin composite restoration.

Because several different steps were involved in our study, including storage, thermocycling, manipulation, and dye penetration, among others, the different results simply may be caused by variation error in any of the aforementioned procedures.[45] Adequate research and data on the microleakage of composite resin restorations after replacing high copper amalgam with nondiscolored dentin is lacking. Harnirattisai and others[25] concluded that the bond strength of discolored dentin after amalgam removal was less than that of normal dentin. However, they did not test microleakage in their study.

The limitations of this study include but are not limited to 1) insufficient *in vitro* data for comparison, 2) artificial aging (thermocycling) without salivary intervention, and 3) dye penetration in a two-dimensional model.

Further research is recommended in which a three-dimensional analysis and thermomechanical loading are used, as these are more analogous to the oral environment. Although clinical trials remain ideal for the evaluation of dental restorations, the results of this study provide scientific evidence for composite resin microleakage after amalgam restoration replacement.

Within the limitations of the study, the following conclusions can be drawn: no significant difference was found between the microleakage of nondiscolored dentin in teeth that were previously restored with amalgam compared with freshly cut dentin, and marginal microleakage in the proximal surface was higher than that in the occlusal surface.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Tufts University School of Dental Medicine.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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