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In Vivo and *In Vitro* Evaluations of Microleakage Around Class I Amalgam and Composite Restorations

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Clinical Relevance

The lining of amalgam restorations showed no significant effect on microleakage around restoration margins. *In vivo* and *in vitro* evaluations confirmed that microleakage was higher in resin composite restorations than in amalgam.

SUMMARY

This study evaluated and compared microleakage values of *in vivo* and *in vitro* placed Class I amalgam restorations with or without three different lining materials and posterior composite restorations with two dentin bonding agents. For the *in vivo* group, 72 standardized Class I cavities were prepared on the occlusal surfaces of molars scheduled for extraction. The test groups (n=12) were: amalgam without lining (A), amalgam with cavity varnish (A+C), amalgam with Clearfil SE Bond (A+CSE), amalgam with Clearfil 2V (A+C2V), composite with Clearfil SE Bond (C+CSE) and composite with Protect Bond (C+PB). The restored teeth were extracted after seven days. The same grouping, materials and techniques were used in 72 extracted molars for the *in-vitro* part of the study. The specimens were immersed in basic fuchsin for 24 hours and sectioned. Microleakage was examined and scored at 20x magnification. Statistical analyses were performed with the Kruskal-Wallis and Mann-Whitney U-tests with the 5% level of significance. Overall, the *in vivo* and *in vitro* test groups were not different from each other. No

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significant differences in microleakage values were observed between the unlined and lined amalgam groups (*p*>0.05). However, since lined amalgam restorations did not reveal any marginal leakage, the application of an adhesive bonding material under the amalgam restorations can be considered. In general, cavity varnish was not as effective as adhesive bonding agents in preventing microleakage in amalgam restorations. Composite restorations demonstrated higher leakage values than amalgam restorations (*p*<0.05), except for A+C (*p*>0.05) in the *in vivo* group. There was no significant difference between the two composite groups for *in vitro* and *in vivo* conditions (*p*>0.05).

INTRODUCTION

Dental amalgam remains the predominant direct material for load-bearing restorations in posterior teeth, and its use continues to be taught at dental schools throughout the world. In the United States, amalgam is still used for approximately 60% of direct posterior restorations.[1] However, the shift to posterior resin composite materials became popular in the mid-1990s. The change from amalgam to composite restorations was most prevalent for one-surface restorations.[1-2]

Dental amalgam has many advantages as a restorative material, because it is strong, durable and easy to use. Some of its disadvantages, however, include its metallic grey color and lack of adhesive properties, making undercuts for mechanical retention necessary. Microleakage has been identified as a significant problem with amalgam due to interfacial gap formation, which may cause tooth discoloration, pulp irritation and secondary caries.[3] Although corrosion products from amalgam alloys may eventually seal the interfacial gap between the tooth surface and the amalgam restoration, numerous investigations regarding microleakage around amalgam restorations have been published over the years.[3-9] These studies suggest that, for the long-term durability of restorations, it is important to prevent or at least control marginal leakage. The microleakage of amalgam restorations may be reduced through adequate cavity preparation, conventional varnish application, use of dentin adhesives, proper amalgam condensation and amalgam burnishing. Numerous studies suggest bonding amalgam with adhesives as an effective way to prevent or minimize initial *in-vitro* microleakage.[6,10-13]

In recent years, resin-based composite restorations have become an increasingly popular alternative to amalgam direct restorations in the posterior dentition, owing to their excellent esthetic and other favorable characteristics. However, posterior composite restorations have also exhibited failures, generally related to excessive wear, tooth sensitivity, polymerization shrinkage, recurrent decay, irreversible pulpitis, open interproximal contacts or restorative material fractures. Raskin and others[14] analyzed the performance characteristics of an early posterior composite in Class I and Class II restorations after 10 years and found a 40% to 50% failure rate. Gaengler and others[15] reported a 74.6% success rate for posterior glass-ionomer cement/composite restorations after 10-year follow-up in a prospective clinical trial. Wilder and others[16] reported a 76% acceptable wear resistance of commercial ultraviolet light-cured composite materials at 17 years. In recent years, the physical properties of commercially available resin composites have been significantly improved, making certain types of resin composites more suitable for posterior stress-bearing areas.[17-20] The advantages of such materials include the reduced need to remove sound tooth substance for retention, favorable esthetic appearance, reinforcement of the remaining tooth substance and increased fracture resistance of the restorative unit. A recent study by Fagundes and others[18] concluded that packable resin-based composites had excellent success and durability during the five-year follow-up.

One may speculate that the microleakage of bonded amalgam or posterior composite restorations may elicit different results under *in vitro* and *in vivo* conditions. *In vivo* studies with up to three years of follow-up revealed no significant differences between bonded and non-bonded amalgam restorations.[21-22] In contrast, in an *in vitro* study using auto radiography, Hersek and others[23] found that amalgam exhibited more leakage than resin composite. However, no study in the literature has evaluated and compared the *in vivo* performance of different bonded amalgam materials and posterior composites by using stereomicroscopy. Therefore, the current study evaluated and compared the microleakage of *in vivo* and *in vitro* placed (1) unlined Class I amalgam restorations, (2) bonded amalgam restorations with two different adhesive bonding agents (Clearfil SE Bond, Kuraray Co, Ltd, Osaka, Japan; Clearfil 2V, Kuraray Co, Ltd), (3) amalgam restorations with cavity varnish (Thermoline, Voco GmbH, Cuxhaven, Germany) and (4) posterior composite restorations with two different self-etching bonding agents (Clearfil SE Bond and Protect Bond, both Kuraray Co, Ltd).

METHODS AND MATERIALS

The current study was conducted in two parts to compare marginal leakage under clinical and laboratory conditions. The current study was approved by the Selcuk University Faculty of Dentistry Ethics Committee. Patients were given a thorough explanation of the nature and scope of the trial and their informed consent was obtained prior to participation (project number: 2006/26-1, 2006/26-2).

In Vivo Evaluation

Seventy-two standardized Class I cavities were prepared in 42 adult patients (ranging in age between 37 and 55 years of age) on the occlusal surfaces of molars scheduled for extraction due to periodontal or prosthodontic reasons. Periodontal reasons included deep periodontal pockets, extensive bone loss and severe tooth mobility. Prosthodontic-based situations related to the periodontal health of the abutment teeth and preparation for a complete denture treatment. The preparations were assigned to one of six study groups, each containing six teeth and 12 cavities. Each restored tooth had an antagonist tooth.

A non-beveled cavity (3 mm in length, 2 mm wide and 2 mm deep) was prepared on the mesial or distal aspect of the occlusal surfaces of each tooth, using a fissure diamond bur (SF-11, Mani Dia-Burs, Mani, Inc, Tichigi, Japan) and a high-speed turbine. The preparations were completed with an inverted cone diamond bur (SI-48, Mani Dia-Burs) at high speed under copious air-water cooling.

During the restorative procedure, the teeth were isolated with cotton rolls and effective saliva suction. Initial cleaning, scaling and temporary splinting of the severely mobile teeth were used to treat periodontally-compromised teeth before restoration. Table 1 lists test materials, lot numbers and the respective manufacturers. The cavities were restored with either amalgam (Cavex Holland BV, Haarlem, The Netherlands) or composite material (Clearfil Photo Posterior, Kuraray Co) according to the following group assignments and manufacturer's instructions.

Group 1–Unlined Amalgam Restoration (A): The amalgam was placed in the cavity using conventional instruments and techniques without any liner application.

Group 2–Amalgam Restoration with Cavity Varnish (A+C): The cavity varnish (Thermoline, Voco) was applied over the prepared area in two separate coats. The first layer of cavity varnish was dried with an air syringe and the second layer was applied and again air-dried. The amalgam restorative material was then placed into the cavity.

Table 1: *Materials Used in This Study and Their Respective Lot Numbers, Ingredients and Manufacturers*

Materials	Lot #	Ingredients	Manufacturer
Amalgam	040914	Ag 45% Ag, Sn 30.5%, Cu 24.0%, Zn 4%	Cavex Holland BV, Haarlem, The Netherlands
Thermoline	23871	1% sodium fluoride, 1% calcium fluoride	Voco GmbH, Cuxhaven, Germany
Clearfil SE Bond			
Primer	00618A	10-Methacryloyloxydecyl dihydrogen phosphate (MDP), 2-Hydroxyethyl methacrylate (HEMA), Hydrophilic dimethacrylate, dl-amphorquinone (CQ), N,N-Diethanol-p-toluidine, Water.	Kuraray Co, Ltd, Osaka, Japan
Bond	00876A	10 MDP, Bis-GMA, HEMA, Hydrophobic dimethacrylate , dl-Camphorquinon, N,N-Diethanol-p-toluidine, Silanated colloidal silica.	
Clearfil Liner Bond 2V			
Primer A	00144B	MDP, HEMA, Hydrophilic aliphatic dimethacrylate, dl-Camphorquinone, Water, Accelerators, Dyes	Kuraray Co, Ltd, Osaka, Japan
Primer B	00142B	Hydrophilic aliphatic dimethacrylate, HEMA, Water, Initiators	
Bond A	00233B	10 MDP, Bis-GMA, HEMA, Hydrophobic dimethacrylate, dl-Camphorquinone, N,N-Diethanol p-toluidine, Silanated colloidal silica	
Bond B	00056C	Bis-GMA, HEMA, Hydrophobic dimethacrylate, Benzoyl peroxide, Silanated colloidal silica	
Protect Bond			
Primer	0012A	Methacryloyloxydodecylpyridinium bromide, Dimethacrylate, solvent (water), initiators, MDP, HEMA, hydrophilic N,N-Diethanol-p-toluidine	Kuraray Co, Ltd, Osaka, Japan
Bond	0020A	MDP, HEMA, Bis-GMA, hydrophobic Dimethacrylate, silanated colloidal silica, initiators, N,N-Diethanol-p-toluidine	
Clearfil Photoposterior	00200A	Bis-GMA, Hydrophobic aromatic dimethacrylate, Triethyleneglycol dimethacrylate, Urethane tetramethacrylate, Silanated silica, Silanated barium glass, Silinated colloidal silica, dl-Camphorquinone	Kuraray Co, Ltd, Osaka, Japan

Group 3–Amalgam Restoration with Clearfil SE Bond (A+CSE): Clearfil SE Bond primer was applied for 20 seconds and dried with an air stream. Next, the dentin-bonding agent was applied. Amalgam was placed after curing the bonding agent for 10 seconds with Elipar 2500 (3M ESPE, Seefeld, Germany) having a light output of not less than 600 mW/cm^2.

Group 4–Amalgam Restoration with Clearfil 2V Amalgam Bonding Agent (A+C2V): Primer A and Primer B were mixed (one drop each), applied to the entire cavity, then left in place for 20 seconds. Subsequently, Bond A and Bond B were mixed and applied to the primed surfaces. The amalgam restoration was placed immediately after the amalgam bonding agent without light-curing.

Group 5–Composite Restoration with Clearfil SE Bond (C+CSE): The liquid primer of Clearfil SE Bond was applied to all of the cavity surfaces with a brush and gently air-dried for 20 seconds. The bonding agent was applied to the primed surfaces and light-cured for 10 seconds using the Elipar 2500 (3M ESPE) curing light. The posterior composite material was then placed in two increments. Each increment was light-cured for 20 seconds.

Group 6–Composite Restoration with Protect Bond (C+PB): The adhesive bonding system Protect Bond was used according to the manufacturer's instructions in the same way as Clearfil SE Bond.

The composite restoration was placed over the bonding agent in two increments and cured, as in group 5.

The teeth were extracted after seven days following the restorations.

In Vitro Evaluation

The exact same grouping and restorative techniques applied to the clinical part of the study were used in 72 extracted human molars. The teeth were stored in distilled water, refrigerated (4°C) and used within three months of extraction. The restored teeth were left in distilled water at 37°C for 24 hours and submitted to thermal cycling for 500 cycles between 5°C ± 20°C and 55°C ± 20°C with 60 seconds of dwell time.[24]

The occlusal surfaces of all the restorations (*in vivo* and *in vitro* groups) were finished and polished. For finishing the composite restorations, diamond finishing burs (Ultradent Products, Inc, South Jordan, UT, USA) were used. Final polishing was performed with Enhance finishing system discs and cups (Dentsply DeTrey, Konstanz, Germany) immediately after light-curing the restorations. The amalgam was triturated at speeds and times prescribed by the manufacturer and condensed into the cavities using an amalgam condenser. Dura-Green finishing stones (Shofu Inc, Kyoto, Japan) and Amalgam polishing kits (Shofu Inc) were used for the amalgam surfaces after 24 hours. All the preparations, restorations and finishing and polishing procedures were performed by the same operator. The restored teeth in the *in vivo* groups remained in the mouth for seven days; they were then carefully extracted.

The root apexes of all the test teeth were sealed with a bonding agent (Clearfil SE Bond, Kuraray, Co, Ltd) and composite material (Clearfil APX, Kuraray Co, Ltd). The entire tooth surfaces were covered with two layers of nail polish, except for the restoration and a 1-mm wide circumferential collar. The specimens were then immersed in 0.5% basic fuchsin for 24 hours and sectioned bucco-lingually with a water-cooled low-speed

Table 2: In Vitro *and* In Vivo *Microleakage Scores Around Amalgam or Composite Restorations of Each Test Group*

Study Groups	**Microleakage Scores**				***p*-values**
	0	**1**	**2**	**3**	
Group 1					
In vitro (n=12)	10	1	1	-	0.580
In vivo (n=12)	11	-	1	-	
Group 2					
In vitro (n=12)	9	1	1	1	0.448
IIn vivo (n=12)	7	2	2	1	
Group 3					
In vitro (n=12)	11	1	-	-	0.317
In vivo (n=12)	12	-	-	-	
Group 4					
In vitro (n=12)	11	1	-	-	0.317
In vivo (n=12)	12	-	-	-	
Group 5					
In vitro (n=12)	3	7	2	-	0.177
In vivo (n=12)	-	9	3	-	
Group 6					
In vitro (n=12)	6	3	2	1	1.000
In vivo (n=12)	6	3	2	1	

Group 1: Unlined amalgam restoration (A), Group 2: Amalgam restoration with cavity varnish (A+C), Group 3: Amalgam restoration with Clearfil SE Bond (A+CSE), Group 4: Amalgam restoration with Clearfil 2V Amalgam bonding agent (A+C2V), Group 5: Composite restoration with Clearfil SE Bond (C+CSE), Group 6: Composite restoration with Protect Bond (C+PB).

$p>0.05$, *statistical evaluation by Mann-Whitney U-test.*

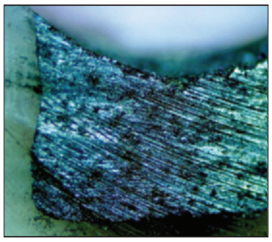

Figure 1. *Representative specimen from Group 1 showing a dye leakage score of 0.*

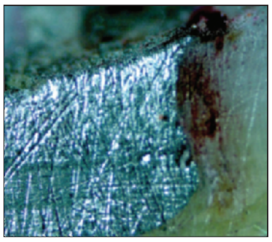

Figure 2. *Representative specimen from Group 2 showing a dye leakage score of 2.*

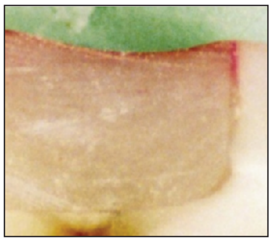

Figure 3. *Representative specimen from Group 5 showing a dye leakage score of 1.*

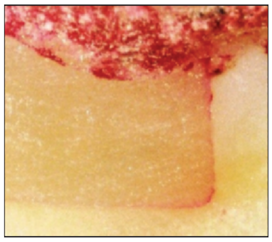

Figure 4. *Representative specimen from Group 6 showing a dye leakage score of 3.*

diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA). Each section was examined under a stereomicroscope (SZ-PT, SZ-40, Olympus Corporation, Tokyo, Japan) at 20x magnification by the same examiner. The extent of microleakage was ranked using the following 0-3 scale at both the enamel and dentin margins of the restorations (Figures 1-4):

0—no dye penetration into enamel

1—dye penetration limited to the enamel of the axial wall

2—dye penetration past the enamel up to the dentin of the axial wall

3—dye penetration past the axial wall involving the floor of the cavity

Data Analysis

The Mann-Whitney U-test was used for comparison of the *in vitro* and *in vivo* measurements. To determine any statistically significant differences between study groups, the Kruskal-Wallis and Mann-Whitney U-tests were conducted. All statistical analyses were performed at the 5% level of significance.

RESULTS

Mean microleakage scores for each group are listed in Tables 2-4. There were no statistically significant differences between the *in vitro* and *in vivo* study groups (Table 2, $p>0.05$). The Kruskal-Wallis test indicated statistically significant differences in microleakage among six *in vitro* (Table 3, $p<0.05$) and *in vivo* (Table 4, $p<0.05$) study groups. Separate statistical analysis of the *in vitro* data with the Mann-Whitney U-test showed no significant differences between unlined amalgams and the A+CSE, A+C2V groups (Table 3, $p>0.05$). However, the A+CSE and A+C2V groups revealed no microleakage at the enamel margins and dentin walls of the cavities for both *in vitro* (Table 3, $p>0.05$) and *in vivo* (Table 4, $p>0.05$) measurements. In contrast, posterior composite restorations placed over Clearfil SE Bond (C+CSE) and Protect Bond (C+PB) demonstrated higher leakage than any of the amalgam groups, except for the amalgam group with cavity varnish for *in vivo* analysis (Table 4, $p<0.05$). There were no significant differences between the A+C group and any of the composite *in vivo* groups (C+CSE and C+PB) (Table 4, $p>0.05$). No significant difference was detected between the C+CSE and C+PB groups ($p>0.05$).

DISCUSSION

The current study evaluated the leakage of several material combinations placed under laboratory (group *in vitro*) and clinical (group *in vivo*) conditions. In the *in vivo* portion of the study, the microleakage scores of the material combinations were investigated after a seven-day period in the oral environment. The rationale for this rather short period was to investigate the early microleakage of amalgam restorations before any corrosion products could be released over time to the cavity/restoration interface. This is important, as the long-

Table 3: *Mean Values and Standard Deviations (SD) of Microleakage Scores with Each Restoration for Each of the Six* In Vitro *Groups*

Study Groups	Mean ± SD	Comparison of the *In Vitro* Groups	*p*-values
Group 1	0.25 ± 0.62	Group 1 vs Group 2	0.569
		Group 1 vs Group 3	0.514
		Group 1 vs Group 4	0.514
		Group 1 vs Group 5	**0.011***
		Group 1 vs Group 6	0.091
Group 2	0.50 ± 1.00	Group 2 vs Group 3	0.248
		Group 2 vs Group 4	0.248
		Group 2 vs Group 5	0.067
		Group 2 vs Group 6	0.286
Group 3	0.08 ± 0.00	Group 3 vs Group 4	1.000
		Group 3 vs Group 5	**0.001***
		Group 3 vs Group 6	**0.023***
Group 4	0.08 ± 0.00	Group 4 vs Group 5	**0.001***
		Group 4 vs Group 6	**0.023***
Group 5	0.91 ± 0.66	Group 5 vs Group 6	0.557
Group 6	0.83 ± 1.02		

Group 1: Unlined amalgam restoration (A), Group 2: Amalgam restoration with cavity varnish (A+C), Group 3: Amalgam restoration with Clearfil SE Bond (A+CSE), Group 4: Amalgam restoration with Clearfil 2V Amalgam bonding agent (A+C2V), Group 5: Composite restoration with Clearfil SE Bond (C+CSE), Group 6: Composite restoration with Protect Bond (C+PB).
**:$p<0.05$, statistical evaluation by Mann-Whitney U-test.*

Table 4: *Mean Values and Standard Deviations (SD) of Microleakage Scores with Each Restoration for Each of the Six* In Vivo *Groups*

Groups	Mean ± SD	Comparison of the *In Vivo* Groups	*p*-values
Group 1 (A)	0.16 ± 0.57	Group 1 vs Group 2	0.074
		Group 1 vs Group 3	0.317
		Group 1 vs Group 4	0.317
		Group 1 vs Group 5	**0.000***
		Group 1 vs Group 6	**0.037***
Group 2 (A+C)	0.75 ± 1.05	Group 2 vs Group 3	**0.015***
		Group 2 vs Group 4	**0.015***
		Group 2 vs Group 5	0.064
		Group 2 vs Group 6	0.775
Group 3 (A+CSE)	0.00 ± 0.00	Group 3 vs Group 4	1.000
		Group 3 vs Group 5	**0.000***
		Group 3 vs Group 6	**0.006***
Group 4 (A+C2V)	0.00 ± 0.00	Group 4 vs Group 5	**0.000***
		Group 4 vs Group 6	**0.006***
Group 5 (C+CSE)	1.25 ± 0.45	Group 5 vs Group 6	0.011
Group 6 (C+PB)	0.83 ± 1.02		

Group 1: Unlined amalgam restoration (A), Group 2: Amalgam restoration with cavity varnish (A+C), Group 3: Amalgam restoration with Clearfil SE Bond (A+CSE), Group 4: Amalgam restoration with Clearfil 2V Amalgam bonding agent (A+C2V), Group 5: Composite restoration with Clearfil SE Bond (C+CSE), Group 6: Composite restoration with Protect Bond (C+PB).

*:$p<0.05$, *statistical evaluation by Mann-Whitney U-test.*

term sealing effects of amalgam restorations seem to be primarily related to amalgam corrosion products, regardless of the type of amalgam or liner used.[25]

The results of the *in vivo* part of the current study demonstrated that the A+CSE and A+C2V groups did not reveal any microleakage. No significant differences were observed among groups 1, 3 and 4. Cavity varnish (group 2) was not found to be as effective as the adhesive bonding systems in preventing leakage of the amalgam restorations *in vivo*. However, groups 5 and 6 were not found to be superior to any of the amalgam groups in controlling microleakage under the applied conditions. In a recent study, Mahler and others [26] confirmed that zinc in amalgam alloys is responsible for the more rapid corrosion of the sealing of amalgams made from zinc-containing alloys. Similar to the current study, they evaluated leakage after one week and observed corrosion products in the occlusal margins of restorations. Therefore, low leakage scores in the amalgam restorations of the current study can be related to corrosion sealing of the zinc-containing alloy used in the study by Mahler and others.

Immediately after packing the amalgam, a rapid contraction may be observed, followed by a slower expansion, then a slight and slow contraction. The net contraction and expansion of an amalgam restoration during setting is defined as ”dimensional change.” Dimensional change is considered negative, if the amalgam contracts, and it is considered positive, if it expands during setting, but ANSI/ADA requires that the dimensional change between five minutes and 24 hours must fall within the range of -15 to +20 µm/cm. High copper alloys show the lowest change.[27] In the current study, the lathe-cut high copper single composition Cavex Avalloy was used. The “dimensional change” was indicated by the manufacturer as +1. This score may be another explanation for low amalgam leakage scores in the current study. Amalgam does not adhere to tooth structure; therefore, a positive dimensional change would result in less gap formation between amalgam and the tooth structure.

Numerous *in vitro* studies demonstrated that bonded amalgam restorations effectively reduced marginal leakage. For example, Belli and others[4] and Amin[28] reported that microleakage values were significantly reduced in lined amalgam restorations when compared to unlined amalgam. Muniz and others[6] observed that a filled adhesive system applied by two methods (light and chemical curing) showed the best performance in the sealing and retention of amalgam restorations.

There is evidence of decreased leakage of fluids with amalgam bonding systems, compared to non-coated or varnish-coated amalgam walls. Al-Jazairy and Louka[9] demonstrated a significant reduction in microleakage around amalgam restorations when Amalgambond Plus or All-V Bond 2 was used as a liner compared to either Copalite varnish or no liner. Dissolution of Copalite varnish was observed over the six-month study period. In addition, Copalite varnish-lined restorations revealed higher microleakage scores than resin-cement lined amalgam restorations. Under most other conditions, however, bonded amalgam restorations performed in a similar way to conventional amalgam restorations with cavity varnish or liner.[29] Morrow and others[3] evaluated the effectiveness of four cavity treatment systems in sealing amalgam restorations and found higher leakage scores with Copalite varnish than with Cervitec, Gluma One Bond or Panavia 21. These materials reduced microleakage to varying degrees, while none of the investigated systems completely prevented microleakage. They did not reveal any statistically significant differences. These findings are very similar to the outcomes of the current study, which showed that the A+CSE and A+C2V groups have less marginal leakage than the A+C group in both *in vivo* and *in vitro* test conditions. In addition, microleakage scores in the A+C group were higher than in unlined amalgams, but not significantly so. The authors of the current study assume that higher leakage scores in the A+C group may be related to the dissolution of cavity varnish during the seven-day *in vivo* period.

El-Housseiny and Farsi[30] compared conventional three-step systems and a single bond adhesive in primary Class V restorations *in vivo* and found that the scores were not different between the two resins. In the current study, C+PB showed a tendency to higher microleakage values than the C+CSE group. However, there was no significant difference between the two. These bonding agents have almost the same composition, except that Protect Bond (PB) contains the antibacterial monomer 12-methacryloyloxydodecylpyridinium bromide (MDPB).[31]

Hersek and others[23] used extracted human teeth and compared microleakage of the different filling materials with the auto radiographic method. These authors demonstrated that amalgam restorations exhibited greater leakage than posterior resin composites, which is not consistent with the results of the current study. In the current study, resin composites revealed greater leakage than most of the amalgam groups (A, A+CSE and A+C2V). Abdalla and Davidson[32] compared the marginal integrity of Class II resin composite restorations under *in vitro* and *in vivo* conditions. Microleakage was observed in all *in vivo* specimens, but only in 60% of the *in vitro* specimens. Barnes and others[33] evaluated Class V resin composite restorations and observed higher leakage scores in the *in vitro* than in the *in vivo* restorations (six-week observation period). In contrast, Hannig and Friedrichs[34] obtained better marginal adaptation of composite restorations under *in vitro*, not perfused, conditions as compared to *in vivo* conditions. The contrasting findings may be due to differences in test conditions, leakage evaluation techniques, cavity types and dimensions, observation times and adhesive/restorative materials. There is an obvious lack of standardized testing parameters undermining the significance and clinical relevance of laboratory leakage studies.[35] In the current study, microleakage scores of *in vivo* and *in vitro* groups were similar for all types of amalgam and composite restorations. This may indicate the adequacy of the applied methods and support the validity and clinical relevance of properly designed laboratory leakage studies.

One of the limitations of the current study is the high level of mobility scores of many of the teeth selected for *in vivo* evaluation. The periodontally-compromised teeth were extracted seven days after placement of the restorations. Therefore, possible effects of masticatory stresses and fatigue load on the marginal integrity of the restorations were probably not sufficiently recorded.

Within the limitations of the current study, it was shown that, overall, unlined and bonded amalgam Class I restorations had lower leakage than bonded composite restorations. These findings support a report by Bagdadi,[36] which concluded that bonded amalgam restorations were more effective in reducing marginal leakage in Class II restorations than resin composites. The great variation of findings among other investigators, however, underscores the need for randomized controlled clinical trials to assess the longevity of bonded amalgam and composite restorations and their effectiveness in reducing marginal leakage.

CONCLUSIONS

Within the limitations of the current study, the following conclusions were drawn:

1) No significant difference between the results of *in vitro* and *in vivo* test groups of the current study suggests that the results with the *in vitro* groups validate the results of the *in vivo* test groups.
2) It was obvious that coating cavity walls with cavity varnish could have no effect on decreasing marginal leakage in amalgam restorations. However, since bonded amalgam restorations did not reveal any marginal leakage, the application of an adhesive bonding material under amalgam should be considered.
3) Resin composite restorations revealed higher leakage scores than amalgam restorations under both *in vivo* and *in vitro* conditions.

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