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Marginal Behaviour of Self-etch Adhesive/composite and Combined Amalgam-composite Restorations

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Abstract - *The aim of this study was to compare the marginal and internal adaptation in self-etching adhesive (SEA) /composite restorations with combined amalgam-resin-based composite restorations in the proximal box with and without bonding agent beneath amalgam both before and after load-cycling. Class II restorations, were manufactured as following a) Bonding agent (Clearfil Liner Bond 2V, Kuraray) beneath amalgam (Tytin, SDS Kerr) and resin-based composite (Clearfil APX, Kuraray) with SEA, b) Amalgam without bonding agent and resin-based composite with SEA and c) Resin-based composite with SEA. Each group divided into two equal subgroups (n=8). Marginal and internal adaptation of first subgroup evaluated after 7-day water storage and of the second after load-cycling in chewing simulator for 1.2x10^6 cycles. Marginal and internal adaptation at cervical and amalgam-composite sites evaluated by videomicroscope and ranked as "excellent"/"non-excellent". Slices of restorations examined under optical microscope to determine the quality of bonding layer. Defects in cervical adaptation observed in the three restorative techniques examined prior loading. Amalgam-composite combination in proximal surface provided comparable marginal and internal adaptation results at cervical wall, to self-etching-composite combination. Portion (25-37.5%) of amalgam-resin-based composite interfaces in proximal box presented no perfect sealing. The application of bonding agent beneath amalgam resulted in relatively inferior cervical adaptation. Loading resulted in fewer excellent restorations in all three restorative techniques but not in a statistically significant level.*

KEYWORDS: bonded amalgam, resin-based composite, load-cycling, marginal adaptation, internal adaptation

INTRODUCTION

The shrinkage stress of resin-based composites developed during the initial setting stages is considered as the main drawback of the materials when used in restorations.[1,2] The phenomenon becomes a clinical issue in restorations with a proximal box, particularly in cases with cervical margins located beneath the cementoenamel junction. The cervical marginal gaps, induced due to the shrinkage, lead to microleakage, one of the most commonly recorded failure reasons in Class II resin-based composite restorations[3]. Although the bonding of the resin-based composites obtained by the adhesive agents may compensate partly the setting stresses [4,5], the perfect marginal adaptation still remains a goal for the resin-based composite restorations.

Various strategies have been introduced to address this shortcoming, like the multi-layer built-up technique [6,7], the use of transparent matrix and wedges [8,9], the apical placement of a chemically polymerized resin-based composite beneath the photo-polymerized material [10], the placement of a layer of unfilled resin beneath the restorative resin-based composite [11].

Additionally, to place a layer of amalgam at the cervical part of the proximal box and afterwards to fill the remaining of the cavity with resin-based composite has been suggested as an alternative method for improvement of cervical adaptation [12]. Recently published clinical evidence of superior clinical performance of amalgam compared to resin-based composites [13] and compomers [14] in challenging oral environment (i.e. children and adolescents) corroborate this treatment concept. The condensability, as well as, the low dimensional changes of amalgam is believed to provide superior marginal adaptation, which could ensure the desired gap-free cervical margins. A variation of this technique is the application of an appropriate and efficient bonding agent beneath the amalgam. The rational for this proposal is the function of the bonding agent as a sealer of the cavity [15] in order to enhance the adaptation of the amalgam.

Regarding amalgam-resin-based composite interface, even though an adequate marginal quality has been recorded immediately after restoration[16,17], in *in vitro* studies, this cannot predict the longevity of the adaptation over the oral service time of the restorations. Remarkable differences between the characteristics of the amalgam and the resin-based composite is possible to affect negatively the initial adaptation achieved.

The self-etching adhesive agents comprise the last category of bonding agents developed for the resin-based composites, which are recommended to be combined with amalgam, as well. These systems induce conditioning and priming of the cavity walls in a single step, claiming the reduction of technical sensitivity presented by the previous generations of adhesive agents and simultaneously, bonding efficiency comparable with the three- and

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two-step bonding systems. Until recently, controversial results concerning their bonding efficiency have been recorded [18-22].

The aim of this study was to compare the marginal and internal adaptation in Class II cavities restored with a combination of amalgam and resin-based composite in the proximal box - with and without bonding agent beneath the amalgam - with corresponding cavities restored with self-etching adhesive and resin-based composite, before and after mechanical load-cycling. The testing hypothesis was that concerning: a) there is no difference, in respect of the marginal and internal adaptation, between combined amalgam-resin-based composite - with and without bonding agent beneath amalgam - and self-etching-resin-based composite restorations and b) the mechanical loading does not influence negatively the quality of marginal and internal adaptation achieved in all restorative systems tested.

METHODS AND MATERIALS

Forty eight (48) caries-free premolars extracted for orthodontic reasons were used for the purpose of the study. Standardized Class II cavities were prepared with one proximal box, parallel buccal and lingual walls and with cervical margins placed 1 mm beneath the cementoenamel junction, using #169 cylindrical carbide burs (Maillefer, Switzerland). The bur was changed after three preparations. The cavities were made with a high-speed turbine under copious water cooling, using a standard cavity preparation device. The dimensions of the occlusal part of the cavities were: 2.5 mm buccal-lingual width, occlusal depth 1 mm beneath the dentin-enamel junction; the dimensions of the proximal box were: 2.5 mm buccal-lingual width and depth 1 mm beside the dentin-enamel junction. The outer margins were not beveled. The materials for the restoration of the cavities are showed in Table 1. The teeth were randomly assigned into three groups (A, B and C) and were surrounded by a metallic matrix (width 7 mm), which was kept stable placing wax along the cervical edge.

In specimens of group A the cavity enamel was etched by 40% phosphoric acid (K-Etchant Gel, Kuraray, Japan) for 10 sec, the primer (mixture of Primer A and B) was applied on the cavity walls and afterwards, the self-cured adhesive (mixture of Bond A and B) was placed. Subsequently, a spherical high copper dental amalgam (Tytin, Sybron-Kerr, USA) was condensed in the proximal box up to 2 mm. After 5 minutes, the bonding agent (Bond A) was applied on the free amalgam surface and the cavity walls and subsequently, photopolymerized for 20 sec with a halogen light-curing unit (Coltolux 4, Coltene, Switzerland, 600mW/cm^2). One 2 mm-layer resin-based composite was placed in the remaining space of the proximal box and finally, the occlusal portion of the cavity was filled. Each layer was photopolymerized for 40 sec.

The teeth in group B were restored with the following procedure: condensation of a 2 mm-layer of amalgam into the proximal box, etching of cavity enamel after 5 min with 40% phosphoric acid (K-Etchant Gel, Kuraray, Japan) for 10 sec, application of the primer (mixture of Primer A and B) on the cavity walls, application of the bonding agent (Bond A) on the free amalgam surface and the cavity walls, photopolymerization for 20 sec and finally, placement of the resin-based composite according to the procedure described in group A.

The teeth in group C were restored solely with bonding agent and resin-based composite. The same procedure was followed as in group B, except of the placement of amalgam. The application of the resin-based composite was made in three, approximately 2mm in thickness horizontal layers, from the cervical towards the occlusal part of the cavity. The restorative procedure for the three groups is presented in Table 2.

The restorations were finished and polished with the Soflex polishing system immediately after photopolymerization of the resin-based composite, to obtain visible outer margins. The amalgam margins of the restorations (group A and B) were polished with rubber polishing cups (Shofu, Japan) The preparation of the cavities and the placement of the restorative materials were performed by one operator.

After finishing of the restorations, each group was divided randomly into two equal subgroups (1, 2). The teeth of subgroups 1 were mounted into moulds with the prepared proximal surface in contact with an intact molar or premolar, immersed into a chamber of distilled water (22±1°C) and exposed to load cycling in a fatigue device (Willytec, Germany) [23]. Teeth of subgroups 2 were stored in distilled water at room temperature (22±1°C) for 7 days and used as control groups for evaluation of internal adaptation.

The protocol for load cycling was as follow: 1.2×10^6 cycles, 1.6 Hz frequency, 49 N load per sample, 2 and 0.3 mm vertical and horizontal movement, respectively. The load was applied at the centre of the occlusal part of the restoration. Ceramic spheres from steatite (Ceramtec, Marktredwitz, Germany) with a radius of 3 mm were used as antagonists.

The adaptation of the restorations was assessed at the outer cervical and amalgam-resin-based composite margins of the proximal surface (marginal adaptation). Afterwards, a section was made through the middle of the restorations, in a distal-mesial direction, using a hard tissue microtome. The one half of each restoration was examined along the cervical-restorative material and amalgam-resin-based composite interfaces (internal adaptation). The measurements performed for marginal and internal adaptation per subgroup are described in Table 3.

The evaluation of marginal and internal adaptation was carried out under a videomicroscope (MS-500c, Moritex, England) at ×100 magnification. The horizontal scanning frequency was 1,560 KHz and the vertical one was 50 Hz. The CCD resolution was 752×582 pixels horizontal and vertical, respectively, while the image resolution was 470 horizontal×520 vertical lines. The videomicroscope observations were conducted under standard illumination of 60 lux.

Previously described assessment criteria were applied for marginal and internal adaptation, distinguishing the "excellent" from the "non-excellent" restorations [24]. Adaptation was assessed as "excellent" when no openings, no gaps, no tooth fracture as well as continuity between resin-based composite-tooth or amalgam-tooth or amalgam-resin-based composite was noticed. Otherwise, the restorations were classified as "non-excellent". Marginal and internal adaptation was graded by one examiner. The results were expressed as the number of restorations ranked as "excellent".

Table 1. *The materials used in the study.*

Material		*Batch no*	*Manufacturer*
Clearfil Liner Bond 2V	*Primer (A and B)* 10- MDP*,2- HEMA*, Hydrophilic dimethacrylate, CQ*, DEPT*, H_2O	A: 00101B B: 00100C	Kuraray, Japan
	Bond A (photopolymerized bonding agent): 10- MDP*, Bis-GMA*, 2- HEMA*, CQ*, DEPT* Silanated colloidal SiO_2 (10 wt %)	00169B	
	Bond B: Bis-GMA*, 2- HEMA*, Hydrophobic dimethacrylate Benzoyl peroxide Silanated colloidal SiO_2	00033C	
Clearfil-APX	Silanated barium glass, silanated colloidal silica,silanated silica, Bis-GMA*, TEGDMA*, CQ*	00852A	Kuraray, Japan
Tytin	Ag 59%, Cu 13%, Sn 28%, Hg 42.5%	5-1339	Sybron-Kerr, USA

**10-MDP: 10-Methacryloyloxdecyl dihydrogen phosphate, 2- HEMA: 2-Hydroxyethyl methacrylate, CQ: Camphorquinone*
DEPT: N,N-Diethanol-p-toluidine, Bis-GMA: Bis-phenol A diglycidylmethacrylate, TEGDMA: Triethylene glycol dimethacrylate

Table 2. *Restorative steps per group.*

Restorative steps / *Groups*	*Etching*	*Mixture of Primer A & B*	*Mixture of Bond A & B*	*Amalgam*	*Etching*	*Bond A*	*Resin-based Composite*
A	✓	✓	✓	✓		✓	✓
B				✓	✓	✓	✓
C	✓	✓				✓	✓

Table 3. *Marginal and internal adaptation measurements per subgroup.*

SUBGROUPS	*MARGINAL*				*INTERNAL*			
	CERVICAL		*AMALGAM-RESIN-BASED COMPOSITE*		*CERVICAL*		*AMALGAM-RESIN-BASED COMPOSITE*	
	*UNL**	*L**	*UNL*	*L*	*UNL*	*L*	*UNL*	*L*
A1	✓	✓	✓	✓				✓
A2					✓			
B1	✓	✓	✓	✓				✓
B2					✓			
C1**	✓	✓				✓		
C2					✓			

** UNL: unloaded, L: loaded.*
*** Amalgam-resin-based composite marginal and internal adaptation was not measured in C1 subgroup.*

Table 4. *Results from the evaluation of marginal adaptation (n=8).*

SUBGROUPS	*MARGINAL ADAPTATION*			
	CERVICAL		*AMALGAM-RESIN COMPOSITE*	
	UNLOADED	*LOADED*	*UNLOADED*	*LOADED*
A1	6* (75 %)	6 (75 %)	6 (75 %)	5 (62.5 %)
A2	-	-	-	-
B1	7 (87.5 %)	6 (75 %)	6 (75 %)	5 (62.5%)
B2	-	-	-	-
C1	7 (87.5 %)	6 (75 %)	-	-
C2	-	-	-	-

** Number of restorations with excellent marginal adaptation and the corresponding percentage (in parenthesis).*

Table 5. *Results from the evaluation of internal adaptation (n=8).*

SUBGROUPS	*MARGINAL ADAPTATION*			
	CERVICAL		*AMALGAM-RESIN COMPOSITE*	
	UNLOADED	*LOADED*	*UNLOADED*	*LOADED*
A1	-	*5 (62.5 %)*	-	*5 (62.5%)*
A2	*6* (75 %)*	-	*6 (75 %)*	-
B1	-	*6 (75 %)*	-	*6 (75 %)*
B2	*7 (87.5 %)*	-	*6 (75 %)*	-
C1	-	*6 (75 %)*	-	-
C2	*6 (75 %)*	-	-	-

** Number of restorations with excellent marginal adaptation and the corresponding percentage (in parenthesis).*

In addition, two, 200 μm in thickness slices were sectioned from two, randomly selected specimens per subgroup, and examined under an optical metallographic microscope (ME 600 Eclipse, Nikon-Kogakou, Japan) at 40x magnification with parallel and crossed polarizers, for the qualitative evaluation of the adhesive layer per interface studied.

The statistical analysis was performed by two-way Kruskal-Wallis non-parametric test, at a=0.05, to define the possible influence of the factors restorative technique and fatigue conditions (loaded-unloaded) on the marginal and internal adaptation at cervical and amalgam-resin-based composite sites. The Statistical Discovery Software JMP (SAS Institute Inc. Version 5.0.1) was used for the statistical analysis.

RESULTS

Representative images from excellent and non-excellent adaptation at cervical and amalgam-resin-based composite sites of the restorations are depicted in Figures 1, 2, 4 and 5. The results concerning the number of restorations with excellent marginal and internal adaptation in all groups are showed in Tables 4 and 5, accordingly.

In unloaded groups, 75 to 87.5 % of the restorations exhibited excellent marginal and internal adaptation at cervical and amalgam-resin-based composite interfaces. Loading reduced in some subgroups, but not in a statistically significant level ($p>0.05$), the number of restorations presented excellent marginal and internal adaptation at cervical and amalgam-resin-based composite areas. No statistically significant differences were detected ($p>0.05$) among the groups filled with the three techniques in terms of marginal and internal adaptation at both sites of the restorations evaluated in unloaded and loaded groups.

The adhesive layer, along the cervical wall of bonded amalgam-resin-based composite restorations, was invisible in the majority of the specimens examined and it was concentrated into the axio-cervical angle (Fig. 3). When it was present on the cervical wall, the adhesive was distributed in a non-uniform layer with the thickest portion located into the axio-cervical angle (Fig. 2).

The most frequent finding at the amalgam-resin-based composite interfaces was an irregular bonding layer with wide range in thickness (Fig. 6), which was obvious even at the outer margin of the proximal surface (Fig. 4). Additionally, the amalgam layer beneath the bonding layer exhibited some deficiencies compared to the underlying amalgam. In a few restorations, such bonding layer was not obvious.

In the resin-based composite restorations, the adhesive agent was evident at the cervical walls of all restorations examined. It was pooled predominantly at the axio-cervical angle, forming a thick layer, in a meniscus shape (Fig. 7).

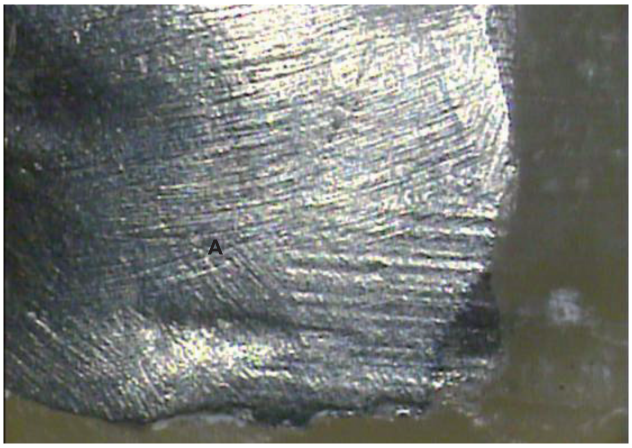

Figure 1. *Excellent cervical marginal adaptation in a restoration with amalgam (without bonding agent) - resin composite combination (Subgroup B1, loaded, videomicroscope 100x).A:Amalgam.*

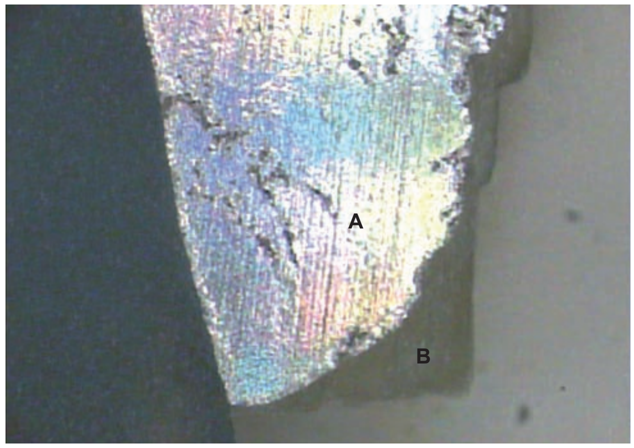

Figure 2. *Perfect cervical internal adaptation in a restoration with amalgam (with bonding agent) - resin composite combination (Subgroup A2, unloaded, videomicroscope 100x).A:Amalgam, B: Bonding agent.*

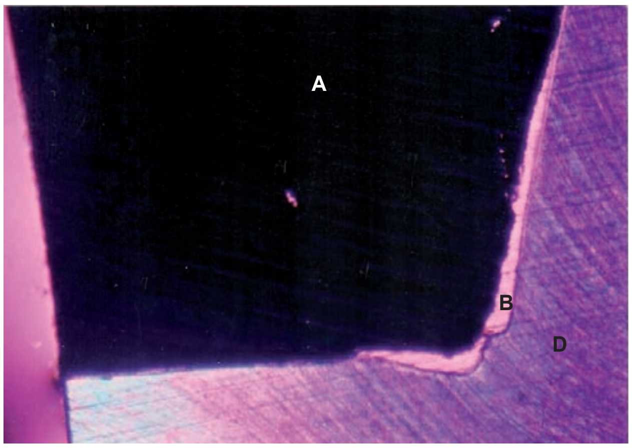

Figure 3. *Cervical interface in a restoration with amalgam (with bonding agent) -resin composite combination.The bonding agent is concentrated into the axio-cervical angle. No evidence of the agent exists along the cervical wall (Subgroup A1, unloaded, optical microscope 40x).A:Amalgam B: Bonding agent, D: Dentine.*

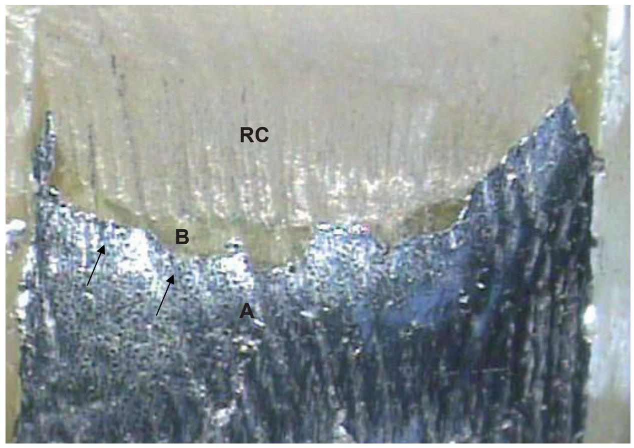

Figure 4. *Excellent amalgam - resin composite margin in the proximal surface.A relatively thick, non-uniform adhesive layer is clearly distinguished between the two materials.The amalgam layer beneath the adhesive presents deficiencies (arrows) (Subgroup B1, loaded, videomicroscope 100x).A:Amalgam, B: Bonding agent, RC: Resin composite.*

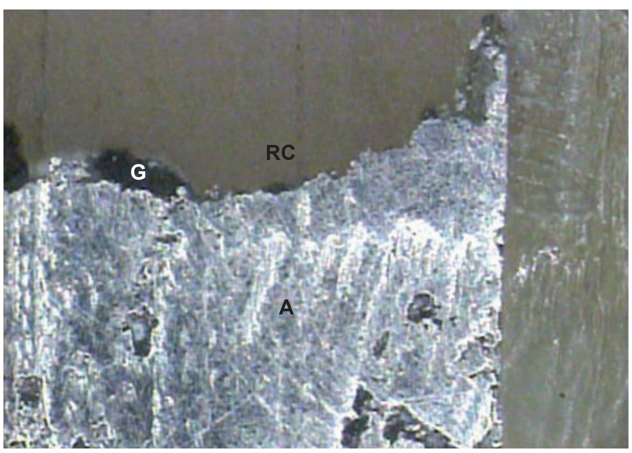

Figure 5. *Non-excellent amalgam - resin composite margin in the proximal surface.The bonding layer can not be clearly observed between the two materials. (Subgroup A1, loaded, videomicroscope 100x). A:Amalgam, RC: Resin composite, G: Gap.*

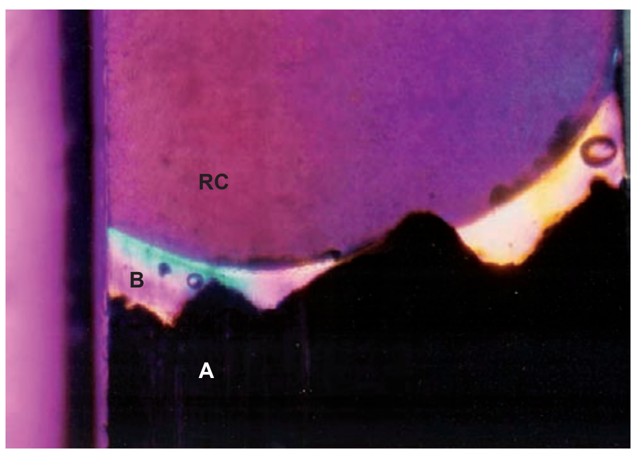

Figure 6. *Amalgam - resin composite interface. Non-uniform in thickness and shape bonding layer between the two material and projections of the amalgam into the bonding layer and the resin composite are noted. (Subgroup B1, loaded, optical microscope 40x). A:Amalgam, B: Bonding agent, RC: Resin composite.*

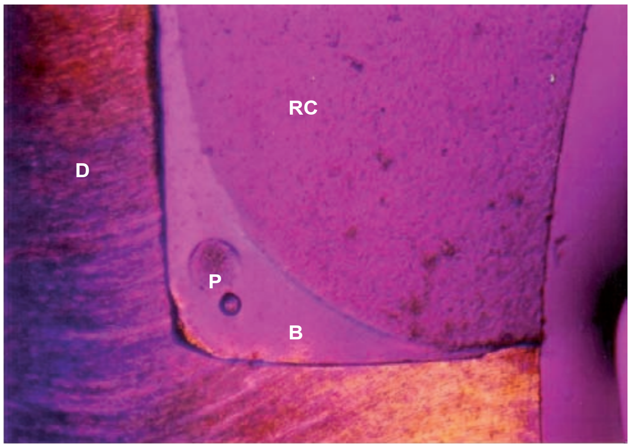

Figure 7. *Cervical interface in a resin composite restoration. A meniscus shape adhesive layer with pore is evident at the axio-cervical angle. (Subgroup C2, unloaded, optical microscope 40x). D: Dentine, B: Bonding agent, RC: Resin composite, P: Pore.*

DISCUSSION

Marginal integrity is an important parameter for the longevity of a restoration. Even though a threshold gap size for development of secondary caries has not been established [25], restorations with any marginal defect fail more frequently than restorations with perfect marginal sealing [26]. In the present study, two of the restorative techniques assessed (groups A and B) comprised restorations with an additional outer margin in the proximal surface, the amalgam-resin-based composite. Openings located at this area could lead to deterioration and possible detachment of both restorative materials over the oral service of a restoration.

The marginal behavior of the restorations can not predict the quality of adaptation at the internal region of the cavity walls and vice versa. Therefore, in the current study, the internal adaptation was also evaluated, since this is considered as a crucial contributor parameter for the long-term clinical performance of a restoration.

The videomicroscope used for the marginal and internal adaptation assessment provides a non-destructive technique, suitable to inspect the interfaces of the restorations and to determine the adaptation according to the criteria applied [27]. Despite these advantages, the videomicroscope operates with limited resolution and sensitivity, because of limited depth field analysis. Thus, an optical metallographic microscopy with parallel and crossed polarizers was utilized for characterization and detailed examination of the adhesive layer at the cavity interfaces. The main drawbacks of this technique are its destructive nature and the need for more than one slice in order to achieve an overview of the specimen. On the other hand, any impression, casting or high-vacuum artifact associated with the replica technique [28] - applied for observation under scanning electron microscopy - is avoided when the examination is undertaken by the polarized microscopy.

According to the manufacturer's recommendations, the self-etching primer adhesive used in the present study can be applied on the cavity walls with no pre-etching of the enamel. However, superior bonding effectiveness for two-step self-primer agents placed on a previously etched with phosphoric acid enamel has been reported [29,30]. In addition, a tendency towards smaller marginal defects has been recorded in a clinical trial when enamel was etched with phosphoric acid prior the placement of a two-step self-etch adhesive [31]. Under these considerations, in the current study, pre-treatment of the enamel by a 40% phosphoric acid for 10s has been incorporated into the adhesive protocol in all groups.

According to the results of the present study the testing hypothesis was confirmed since no statistically significant difference ($p>0.05$) was found neither among the three filling procedures followed nor after experimental loading of the restorations.

It was an important finding that a portion of restorations, before subjected load-cycling and regardless the restorative material (amalgam or resin-based composite) exhibited defects in initial adaptation at the cervical margins. Another interesting aspect of the results was the relatively inferior, but not in a statistically significant level, cervical marginal and internal adaptation of the amalgam when combined with the bonding agent, as elsewhere stated [16].

The reduction of friction when the amalgam is condensed against a cervical wall covered with the bonding agent may be a contributor factor for the incidence observed. Bonding agent between amalgam and cavity walls has been suggested to function as an intermediate adhesive layer [15,32]. It is recommended the amalgam to be condensed against unpolymerized adhesive agent in order to produce interlocking projections into the adhesive for mechanical retention [33], as both the alloy and the resin-based composite start setting [34]. It is therefore preferable a chemical-cured adhesive to be applied, for efficient polymerization under the amalgam [35] and for simplified intermixing with the slow hardening agent [33]. The mechanical intermingling of adhesive and amalgam is related to adhesive thickness and, primarily, to how thick the unpolymerized resin layer is before amalgam insertion [35,36]. However, the unset adhesive can be displaced under the condensation pressure of amalgam causing uneven distribution of the adhesive agent beneath amalgam and variability of the adhesive layer [35]. It can explain the concentration of the adhesive agent into the axio-cervical angle and the disappearance along the cervical wall, as observed in specimens of the current study.

The amalgam-resin-based composite combination in the proximal box creates an additional interface for the restoration, which presented more accentuated marginal and internal defects than the cervical site, especially after loading. The photopolymerized adhesive layer (Bond A) placed between amalgam and resin-based composite was thick, clearly distinguishable in most of the specimens. Gaps were located either below or above the bonding layer at the amalgam-resin-based composite interface.

Fresh amalgam is proposed to dispose a high critical surface tension compared to other restoratives [37] which affects negatively the wettability of the surface [15], a decisive factor concerning bonding quality [38]. The irregularities of the amalgam surface could also lead to air entrapment and subsequently to bonding inconsistency. As a sequence, the bonding agent can not effectively function as an intermediate layer when placed above amalgam. Furthermore, pooling of the adhesive agent at the internal angles has been reported [32] and is possible to provoke a negative bonding effect [39].

Generally, pooling of the bonding agents at the internal angles is likely to occur in a three-dimensional cavity preparation and represents a more relevant clinical situation[40]. A viable solution could be the combination of the two materials without the application of a bonding agent on the free amalgam surface prior the resin-based composite placement [16,17]. Additionally the application of different surface conditioning techniques on amalgam could provide superior bonding results [41].

Moreover, the inspection of the amalgam-resin-based composite interfaces revealed that the quality of the amalgam beneath the bonding agent seems to be inferior compared to the bulk amalgam. It may be attributed to incorporation or and diffusion of the unset adhesive agent into the amalgam during its application procedure.

The photopolymerized Clearfil Bond Liner 2V applied on the cavity walls in combination with the resin-based composite (group C) formed a relatively thick bonding layer. Since it is difficult to obtain a thick layer without defects, pores have been observed into the layer at the internal axio-cervical angle of several composite restorations, which may influence the strength of the adhesive layer. The absence of solvent and the silica nanofillers (wt. 10 %) contained should account for the thickness of Clearfil LB2V layer [42]. The thick layer of the Clearfil Bond Liner 2V has been reported to increase its bond strength [42] and the system has been showed to dispose one of the highest microtensile bond strength on dentin compared to other systems [43]. Nevertheless, in this study, a small portion of restorations exhibited marginal and internal defects, prior and after the load-cycling.

Mechanical loading, under the experimental conditions applied did not considerably influence the marginal and internal adaptation of the resin-based composite restorations tested, although, Nikaido et al. [44] reported reduction of bond strength, assessed by microtensile, in Class I restorations, after a combination of thermal and mechanical loading. The results of the present study may indicate that to achieve an initial perfect adaptation by the restorative combinations tested is more difficult than the bonding endurance over fatigue.

Under the limitations of the current study, it seems that the combination of amalgam and resin-based composite in the proximal box of a Class II resin-based composite restoration was not able to improve the quality of adaptation along the cervical wall, which is considered as the weak site of a resin-based composite restoration. Consequently, from a clinical approach, this technique should be characterized as time-consuming, technically sensitive, and simultaneously, cannot offer advantages over the combination of a resin-based composite with a self-etch primer adhesive. For the same reasons, the combination of an amalgam with a bonding agent should be avoided because it causes rather inferior adaptation results.

CONCLUSIONS

Defects in cervical adaptation were revealed in the three restorative techniques tested prior the load-cycling. The combination of amalgam and resin-based composite in the proximal surface of a Class II resin-based restoration provided comparable results, regarding cervical marginal and internal adaptation, with the combination of resin-based composite and self-etch primer adhesive.

The application of a bonding agent beneath the amalgam resulted in relatively inferior cervical adaptation. Loading of the restorations did not significantly reduce the number of restorations with excellent adaptation in the three restorative techniques and at the interfaces examined.

ADDRESS FOR CORRESPONDENCE

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