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The Efficacy of Immediate Dentin Sealing Techniques on Marginal Micro Leakage of Composite Resin Inlays

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Authors' contributions

This work was carried out in collaboration between all authors. Author AMS designed the study and wrote the first draft of the manuscript. Author LGO performed the experiments and managed the literature searches. Authors GAB contributed substantially to experimental design and discussion. Author LHBJ performed the statistical analysis and co-wrote the manuscript. All authors read and approved the final manuscript.

Article Information

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ABSTRACT

Aim: To evaluate the influence of two immediate dentin sealing (IDS) techniques on the marginal microleakage of composite inlays.
Methodology: Thirty human third molars received MOD preparations, with the mesial proximal box extending above and the distal box extending below the cemento-enamel junction. The teeth were randomly divided into groups (n=10): G1 – control, without IDS; G2 – IDS with Clearfil SE Bond; G3 - IDS with Clearfil SE Bond and Protect Liner F. Impressions of the preparations were made, followed by provisional restoration. ED Primer A and B were applied to the preparation followed by

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Panavia F for the luting procedure of the composite inlays. The samples were thermocycled 2500x between 5°C and 55°C, then placed in a 0.5% methylene blue dye solution for 24 hours. The samples were sectioned in the mesio-distal direction and evaluated for microleakage at the mesial and distal margins under a stereo microscope at 20x magnification. The dye penetration was scored as 0 (no penetration), 1 (penetration to half of the gingival wall), 2 (penetration along the gingival wall), 3 (penetration extending into the axial wall).

Results: According to Kruskal-Wallis analysis (α=.05), there were no significant differences in the dye penetration between the groups in either the enamel (*P*=.07) or the dentin (*P* =.31). According to Wilcoxon, the dye penetration in the dentin was higher than in the enamel (*P* =.03) for group 3.

Conclusion: The IDS with Clearfil SE Bond, either associated or not associated with Protect Liner F, was not capable of producing complete sealing of the enamel and dentin margins.

Keywords: Enamel; dentin; immediate dentin sealing; microleakage.

1. INTRODUCTION

Composite resins have been largely used in Dentistry for aesthetic reasons. Polymerization shrinkage is an intrinsic characteristic of these materials [1,2]. Shrinkage can lead to marginal microleakage [3,4] when the forces generated during the polymerization cause the rupture of the adhesive-tooth interface [5]. When an indirect technique is used to restore a tooth with composite resin, the polymerization shrinkage occurs outside the tooth, and the possibility of microleakage is reduced [6,7].

The traditional technique used for producing indirect restorations consists of making an impression of the tooth immediately after preparation and luting an acrylic resin restoration with provisional cements, or with provisional resin materials applied directly to the prepared tooth. Once the permanent restoration is ready, the provisional material is removed and an adhesive system is applied to the tooth, followed by a resin cement for the adhesive luting procedure.

Studies have shown that adhesive systems bond better to freshly cut dentin compared with dentin contaminated with temporary materials [8,9] and that this contamination may cause microleakage [10]. To avoid this problem, the immediate dentin sealing (IDS) technique was suggested in the early 1990s [11]. This technique consists of the application of an adhesive system immediately after finishing the tooth preparation but before the impression. Another technique consists of the application of an adhesive system and a low-viscosity composite resin to the dentin immediately after finishing the preparation [12,13]. It is believed that a layer of low-viscosity composite resin helps to protect the hybrid layer and, consequently, preserve the dentin seal [14].

The clinical advantages of the IDS technique are related to the seal and the protection of the dentin-pulp complex immediately after cavity preparation by the application of a resin agent, preventing and decreasing the sensitivity and bacterial infiltration during the provisional stage [15,16].

Studies have shown that there is an increase in the bond strength when the IDS technique is applied [17-23]. However, there is no consensus about the correlation between the bond strength and the level of microleakage [24,25]. Therefore, it is important to evaluate how the IDS techniques relate to the marginal sealing ability.

The aim of this study was to evaluate the influence of two IDS techniques on the microleakage of composite resin inlays. This study was conducted under the null hypothesis that IDS techniques do not influence the microleakage of composite resin inlays.

2. MATERIALS AND METHODS

Thirty sound third molars were obtained from the Tooth Bank of the Pontifical Catholic University of Rio Grande do Sul after Ethics Committee approval was obtained (n. 0118.0.002.000-10). The teeth were cleaned and disinfected in 0.5% chloramine for 24 h, then stored in distilled water at 4°C. The buccal-palatal and mesio-distal dimensions of each tooth were measured with a digital caliper rule (Mitutoyo, Suzano, SP, Brazil). A variation of 0.5 mm was allowed for each measurement to standardize the dimensions of the teeth.

The teeth were randomly divided into three groups (*n* =10): group 1, conventional technique (control); group 2, IDS with the adhesive system; group 3, IDS with the adhesive system and a

low-viscosity composite resin. The materials used are listed in Table 1.

2.1 MOD Preparation

Each tooth was mounted vertically in a plastic ring with auto polymerizing resin (Jet Classico, São Paulo, SP, Brazil) up to 2 mm below the cement-enamel junction (CEJ). A single operator performed cavity preparations on the mesial, distal and occlusal surfaces with a 4159 diamond bur (KG Sorensen, Barueri, SP, Brazil) at a high speed under constant water and air cooling. The width between the buccal and lingual cavosurface angle was two-thirds the distance between the buccal and lingual cusp tips, and the occlusal isthmus was 3 mm deep. The widths of the proximal boxes corresponded to one-third the distance between the buccal and lingual surfaces of the teeth at the level of the gingival wall, and 1.5 mm deep. The mesial boxes were located 1 mm coronal to the CEJ, and the distal boxes were located 0.5 mm below the CEJ. The internal line angles were rounded, the cavosurface angles were approximately 90° and the angle of divergence of the walls of the preparations was approximately 6°. The dimensions of the cavity were standardized using a digital caliper (Mitutoyo, Suzano, SP, Brazil) with a precision of 0.01 mm.

2.2 IDS Techniques

Group 1 (the control) received only the cavity preparation. In group 2, the Clearfil SE Bond adhesive system was applied immediately after the preparation as follows: the self-etching primer was applied to the dentin using a brush tip and was left in place for 30 s. Excess solvent was removed by air drying for 5 s. The bond was applied to the surface cavity with a brush tip, and gentle air drying was applied for 3 s. The tip of the light-curing unit (Optilux Plus, Gnatus, Ribeirão Preto, SP, Brazil) was positioned at the top of the occlusal surface of the cavity preparation, followed by light-curing for 20 s. The light intensity was controlled by a radiometer (Model 100 Demetron, Kerr, Danbury, CT) between 450 and 500 mW/cm^2. Polymerization of the adhesive was followed by the application of an air-blocking barrier (glycerin jelly) and 10 s of additional light curing to polymerize the oxygen inhibition layer [20]. In group 3, Clearfil SE Bond was applied as described in group 2 without the air-blocking barrier. After application of the adhesive, Protect Liner F was placed on the adhesive surface using a brush-on technique and light-cured for 20 s. The surface of the cured flowable composite resin was wiped with a cotton pellet soaked in alcohol for 10 s to remove the unpolymerized layer on the surface [26].

Table 1. Materials used in the study

Materials	Composition	Batch number	Manufacturer
Clearfil SE Bond	*Self-etch primer*: 10-MDP, HEMA, hydrophilic dimethacrylate, photo-initiator, water. *Adhesive*: 10-MDP, bis-GMA, HEMA, hydrophilic dimethacrylate, microfiller	51436	Kuraray Medical Inc., Tokyo, Japan
Protect Liner F	TEG-DMA, Bis-GMA, methacryloyl fluoride-methyl, methacrylate copolymer	00069A	Kuraray Medical Inc., Tokyo, Japan
Panavia F	*ED primer A*: HEMA, 10-MDP, 5-NMSA, water, accelerator	00262A	Kuraray Medical Inc., Tokyo, Japan
	ED primer B: accelerator, water, sodium benzene sulfinate	00137A	
	A-Paste: Methacrylate, 10-MDP, quartz-glass, microfiller, photoinitiator	00362A	
	B-Paste: Methacrylate, barium glass, sodium fluoride, chemical initiator	00065A	
Filtek Z250	Bis-EMA, UDMA, Bis-GMA, TEGDMA, silicon dioxide and zirconium dioxide fillers	5CG	3M ESPE, St. Paul, MN, EUA

HEMA=hydroxyethylmethacrylate; TEGDMA= triethylene glycol dimethacrylate; UDMA= urethane dimethacrylate; Bis-GMA= bisphenol-glycidyl methacrylate; 10-MDP = 10-methacryloyloxydecyl dihydrogen phosphate; Bis-EMA: ethoxylated bisphenol A dimethacrylate; 5-NMSA: N-methacryloxyl-5-aminosalicylic acid

2.3 Restorative Procedures

Impressions of the preparations were taken with polyvinyl siloxane (3M Espe, Saint Paul, MN, USA) with individual trays made from self-cured acrylic resin using the putty/wash one-step technique. The impression material was allowed to set for 10 min before it was removed from the preparation. Temporary self-cured acrylic resin crowns were then luted onto the preparations with non-eugenol cement (Temp Bond NE, Kerr, West Collins, CA, USA). Tooth specimens were stored in water at 37°C water for 7 days. The impressions were poured after 1 h using Durone Type IV stone (Dentsply, York, PA, USA).

The composite resin Filtek Z250 (3M Espe, Saint Paul, MN, USA) was used to build the composite inlays. The casts were lined with a die spacer, except in the marginal areas. Four horizontal layers of composite resin were inserted into the casts with a Thompson spatula (nos. 2 and 12), producing a 90° inclination between the internal slopes and the cusps. Each resin layer was light-cured for 40 s, followed by finishing with polishing discs and silicone tips (Soft-Lex, 3M Espe, Saint Paul, MN, USA).

2.4 Luting Procedures

Following storage, the provisional restorations were removed, and the remaining temporary cement on the inlay preparation was scraped off with a dental instrument. Subsequently, the dentin (group 1) and sealed dentin (groups 2 and 3) were cleaned with a mixture of water and pumice using a rotary brush for 10 seconds. The fitting surface of the composite inlay was cleaned with alcohol and sandblasted with 50 µm aluminum oxide for 5 s, rinsed and dried. A layer of silane (Ceramic Primer, 3M ESPE, St. Paul, MN, USA) was applied, followed by gentle air-drying for 5 s. The coated surfaces of the preparation (except group 1) were then acid-etched with 37% phosphoric acid for 10 s and rinsed and dried to remove debris. A mixture of ED Primer A and B was applied for 30 s and gently air-dried for 5 s. The base and catalyst of the Panavia F resin cement were mixed according to the manufacturer's instructions. The crowns were seated using a 1 kg standard load for 2 min. Excess cement was removed with a microbrush and each surface (buccal, lingual, mesial, distal and occlusal) was light-cured for 40 s. The margins were finished with polishing discs (Sof-Lex). The specimens were stored in distilled water at 37°C for 24 h.

2.5 Microleakage Evaluation

After storage, the specimens were thermocycled in water for 2500 cycles between 5 and 55°C, with a dwell time of 30 s. After the thermocycling, the teeth were covered with nail varnish, except for the restoration and a 0.5 mm margin around it. The crowns were immersed in a 0.5% methylene blue dye solution for 24 hours at 37°C. After the thermocycling and dye penetration steps, the teeth were rinsed thoroughly with water and sectioned in the mesio-distal direction, in the center of the crowns, with a diamond disc mounted on a low speed laboratory cutting machine (Labcut 1010, Extec Corp., London, UK) with water cooling. The cut surfaces were polished with 1000-, and 1200-grit silicone carbide abrasive papers and wetted with water using manual pressure and rotary movements. Sectioned restorations were examined under a stereomicroscope at 20x (Olympus Corp., Tokyo, Japan). The extent of the dye penetration in the mesial gingival wall (enamel) and distal gingival wall (dentin) was evaluated by one experienced examiner and recorded as follows: 0= no penetration; 1= dye penetration to half of the gingival wall; 2= dye penetration along the gingival wall; 3= dye penetration extending into axial wall.

2.6 Statistical Analysis

The data were analyzed using the Kruskal-Wallis non-parametric test to compare the microleakage between the groups. The Wilcoxon non-parametric test was used to compare the microleakage between the enamel and dentin. The significance level was 5%.

3. RESULTS

According to Kruskal-Wallis non-parametric test, there were no significant differences in the microleakage scores between the groups, for either the enamel (P =.07) or the dentin (P =.31). Fig. 1 shows the microleakage score distribution in the groups.

According to the Wilcoxon non-parametric test, there were significant differences in the microleakage scores between the enamel and dentin for group 3, with the microleakage scores in the dentin statistically higher than those in enamel (P=.03). There were no significant differences in the microleakage scores between the enamel and dentin for group 1 (P =.38) and group 2 (P =1.00).

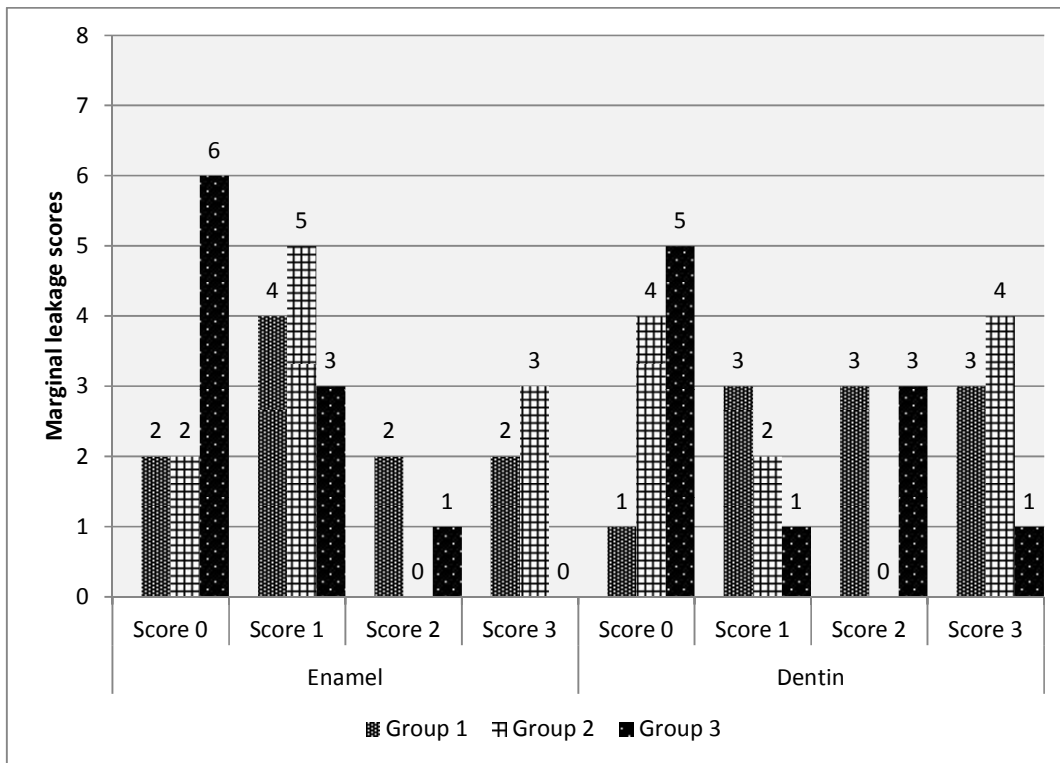**Fig. 1. Microleakage scores distribution in the groups**

4. DISCUSSION

In the literature, microleakage has been defined as the passage of bacteria, fluid, molecules or ions that are not "detectable clinically" between the cavity wall and the restorative material [27]. Microleakage can occur due to the deterioration of the tooth-restoration interface, differences in the thermal expansion coefficients of the tooth and restorative material, or polymerization shrinkage [28]. The clinical consequence is that the higher the marginal microleakage, the greater the chances that fluids, ions and bacteria percolate through the tooth-restoration interface [29]. Microleakage causes dissolution of luting materials [30], which can result in bacterial contamination and post-operative sensitivity, and can even compromise the pulp vitality [29]. Therefore, it is important to use materials and techniques that prevent or reduce microleakage.

According to the results obtained in the present study, the null hypothesis was accepted because there were no significant differences in the microleakage between the three different procedures related to adhesive luting, either in the enamel or dentin. Fig. 1 shows that any technique could avoid marginal microleakage,

which was consistent with the results of prior studies [23,31].

In the present study, the Clearfil SE Bond adhesive system was applied to the dentin immediately after the tooth preparation in groups 2 and 3. In group 1 (conventional technique), ED *Primer* was applied to the dentin after the provisional restoration removal, followed by Panavia F resin cement application.

ED Primer is a one-step self-etching primer that has a moderate capacity of dentin demineralization. Due to the presence of the hydrophilic monomer HEMA, ED Primer presents some permeability, allowing changes at the dentin–adhesive interface, and consequently, hydrolytic degradation of this interface [32]. It was suggested that the application of hydrophobic adhesive over the ED Primer layer before luting with Panavia F could decrease the amount of microleakage [32]. Clearfil SE Bond is a two-step adhesive system with a pH close to 2. The system has a self-etching primer and an adhesive and a moderate capacity to demineralize dentin [33]. As the primer in this adhesive system also has the hydrophilic monomer HEMA, it has some permeability.

However, the application of the adhesive on the primer, which contains a larger quantity of hydrophobic monomers, tends to reduce the permeability of this adhesive system [34]. Therefore, the expected result of the present study was to obtain less microleakage in group 2 relative to the control group, which was not confirmed. The differing material composition may partially explain this finding.

The ED *Primer* and Clearfil SE Bond adhesive system contain the acidic monomer 10-MDP, which chemically bonds to the calcium of the hydroxyapatite that remains partially attached to collagen [35], promoting a hydrolytically more stable dentinal bond. This chemical bond may favor a similar protection effect on the hydrolytic degradation for both adhesive systems, resulting in microleakage scores without significant differences between them.

Despite the absence of significant differences between the groups due to the homogeneous microleakage scores distribution, there was a trend towards more scores of 0 and fewer scores of 3 when the Clearfil SE Bond adhesive system and the Protect Liner F low-viscosity composite resin were applied to the dentin immediately after cavity preparation (group 3). It is possible that the combination of various factors may have contributed to this finding. For example, perhaps additional polymerization on the Clearfil SE Bond at the moment of low-viscosity composite resin photo activation occurred, favoring a higher rate of monomer-to-polymer conversion in the adhesive most closely in contact with the tooth substrate [32]. This higher rate of conversion may reduce the permeability of the adhesive, resulting in a greater durability of the adhesive interface [36]. In addition, the higher rate of conversion may favor a higher adhesive bond strength to the dental substrate, as this an important factor in resisting the polymerization shrinkage that is restricted to the thin layer of Panavia F resin cement [37]. Studies show that polymerization shrinkage stress that is generated due to the lack of non-adhered surfaces can break the bond between the resinous material and the cavity walls, resulting in gaps and failures in the interfaces [38,39]. Therefore, the bond of the adhesive to the substrate is important in resisting the shrinkage stresses that are generated during polymerization. In addition to these factors, the adhesive was applied directly over the cut dentin and was not contaminated with the temporary material, thus favoring a better bond [8,9]. All of these factors

may have influenced the microleakage values in group 2 because the Clearfil SE Bond also received further polymerization at the time the hydrophilic gel was applied. However, the difference between groups 2 and 3 is the application of low-viscosity composite resin, which may have favored a greater absorption of the stresses generated by the polymerization shrinkage of the resin cement, contributing to an increased relief of polymerization shrinkage in the adhesive interface [38,40].

In both IDS techniques, the bond of the cementing agent to the pre-existing resin layer should be promoted by cleaning the surface prior to cementation [41] to remove remnants of the provisional cements that can cause a significant reduction in the bond strength of the luting agent [42,43]. Therefore, after removing the provisional restoration, the preparations in all of the groups received prophylaxis with pumice and water.

Although the techniques of IDS have the primary purpose of sealing the dentin, this study also evaluated the microleakage in the enamel. This is because, depending on the clinical case, the preparation margin may be in enamel in the cervical proximal region. Even when the clinician seeks to apply the adhesive system only to the dentin, the enamel is usually also treated.

Comparing the microleakage between the enamel and dentin, there was a significant difference between these substrates only in group 3, in which the scores in the dentin were significantly higher than those in the enamel. Despite the lack of significant differences between the enamel and dentin for groups 1 and 2, there was a trend towards scores of 2 and 3 when assessed in dentin. This finding is in agreement with the literature, which shows that microleakage tends to be higher in dentin than in enamel [44,45]. The dentin has a higher biological variability than enamel, making it a more difficult substrate to obtain a stable bond [46]. Possibly, the bond to dentin did not withstand the interfacial stresses generated by the polymerization shrinkage of the resin cement. Another possible explanation for not observing lower microleakage scores in the enamel in relation to the dentin is the fact that ED *Primer* and Clearfil SE Bond have questionable bonding to enamel as they have a moderate level of demineralization capacity. In the case of Clearfil SE Bond, laboratory studies have reported an equal or lesser effectiveness of the bond to enamel compared to adhesive systems that employ phosphoric acid etching [47,48].

This study used thermal cycling as the aging feature of the samples to simulate the degradation of bonded interfaces that occurs over time in the oral cavity. The effectiveness of thermal cycling on microleakage as a model to simulate clinical aging has been a subject of controversy among researchers [49,50]. Although there has been discussion on the validity of thermal cycling as a valid model system, it has been frequently utilized in microleakage studies.

The transfer of results from laboratory studies to the clinical setting must be performed with caution, as *in vitro* studies can never fully reproduce the conditions and behavior of the oral cavity behavior. According to the results, it seems that IDS with Clearfil SE Bond, either alone or in combination with Protect Liner F, does not prevent microleakage. However, longitudinal clinical studies are needed to confirm these results in indirect MOD restorations.

5. CONCLUSION

This study showed that an IDS technique with the Clearfil SE Bond adhesive system, either associated or not associated with the Protect Liner F low-viscosity composite resin, does not produce complete sealing of the margins in enamel or dentin.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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