Marginal Leakage in Interfaces Formed by Bovine Dentin and Adhesive Cements Applied with Different Bonding Techniques

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ABSTRACT

Objectives: The aim of this in vitro study was to evaluate the marginal microleakage in interfaces formed by bovine dentin and adhesive cements applied with different bonding techniques.

Materials and Methods: Thirty bovine teeth dentin blocks measuring 5 mm \( \times \) 5 mm \( \times \) 1 mm were made with one side completely in dentin substrate (20 mm³). The dentin blocks were randomly divided into three groups according to the cementation technique used: CTS+RX (conventional 3-step adhesive + dual-activation resin cement), SBS+RX (single-bottle self-etching adhesive + dual-activation resin cement), and SAC (self-adhesive cement). A photopolymerizable composite resin block with the same dimensions was fixed on the dentin block according to the instructions for each adhesive technique. The dentin-composite resin blocks made with each adhesive technique were separated into subgroups (\( n = 5 \)) for 7 days (control) and for 6 months of water storage. After each period, the blocks were individually immersed in test tubes containing neutral methylene blue dye for 2 hours. The samples were washed, dried, and evaluated with a stereoscopic magnifying glass, and the amount of infiltrated pigment was analyzed using spectrophotometry (Beckman DU 65). The data were submitted to two-way ANOVA and Tukey’s test (5%).

Results: Microleakage level for the conventional 3-step or self-etching technique remained similar for 7 days (18 ± 25 and 43 ± 45 \( \mu \)m, respectively) or 6 months (42 ± 55 and 52 ± 87 \( \mu \)m, respectively). The self-adhesive technique showed higher microleakage levels for 7-day (263 ± 98 \( \mu \)m) and 6-month periods (441 ± 226 \( \mu \)m) compared to other adhesive techniques. There was a statistically significant difference between evaluation times only for the self-adhesive technique.

Conclusions: Higher microleakage levels by storage in water occurred with the self-adhesive technique in both evaluation periods.

Clinical Relevance: The different microleakage levels promoted at the dentin-composite resin interface should be considered in adhesive clinical procedures in relation to long-term use.

Keywords: Adhesive cementation, microleakage, Resin cement, water storage.

1. Introduction

The clinical success related to the bonding procedures of indirect dental restorations is directly related to the material, technique employed for luting, marginal sealing of the interface, and bond level between tooth and restoration, while the adhesion to enamel appears to be a weak link in the bonding procedure [1]. Furthermore, a previous study showed that self-adhesive resin cements provided a much better marginal seal for noble alloy full-cast crowns compared to resin-modified glass ionomer or dual-cured resin-based cements [2].

It has been claimed that one of the problems commonly found in indirect dental restorations is microleakage at the interface between the tooth and restorative material due to insufficient marginal sealing. This phenomenon
is characterized by the penetration of fluids, molecules, ions, and bacteria being clinically imperceptible. However, a previous study showed that no association was observed between microleakage and marginal gap. The luting agents investigated revealed different sealing abilities, and the differences were not associated with specific types of materials [3]. Moreover, the integrity of the marginal seal was reported as a primary factor for the clinical success of this type of restoration, since the dental cements revealed different bonding abilities, and the resin cement resulted in a percentage of zero microleakage scores [4].

The maintenance of the marginal sealing of the cement to tooth structure is susceptible to critical situations in the oral environment. In a clinical situation, the adhesive interface remains in a humid environment due to the constant presence of saliva, which plays a negative role in maintaining the bond. Time has a significant influence on the sorption and solubility behavior of the composite resin, and the mass increase and/or decreases depending on the pH of the oral solution [5]. A previous study showed that storage in distilled water can simulate interface degradation over time. Therefore, significant differences were observed in microleakage in restorations of different restorative resin composite materials submitted to thermocycling and immersed in 0.5% fuchsin for 24 hours. When compared to the water storage in the 24-hours or 6-month intervals, the microleakage was higher for 6 months. However, the number of restoration horizontal layers (2 layers vs. 4 layers) did not result in any differences in microleakage scores [6]. The occurrence of microleakage at the interface has been related to dental hypersensitivity, secondary caries, and pulpal complications. A study revealed that the quantity of bacteria filtered from the base of Class V cavity restorations was directly related to the type of material used. Each material produces a varying number of bacterial colonies, and the histopathology of the pulp correlates directly to the microbiological data [7]. Another interesting fact is that the vitality and dentine repair capacity of the pulp is dependent on odontoblast survival [8], which would also be injured by the marginal microleakage.

It has been claimed that the level of microleakage is also directly related to the cement types. Although this occurrence has yet to be verified in all dental cements, in vitro studies have shown that resin cements have lower microleakage levels [2], [3]. In that regard, the adhesive composite resin luting system showed clinically acceptable marginal discrepancies and an excellent ability to minimize microleakage in all-ceramic crowns [9].

Conventional resin cementation is based on adhesive polymeric systems and can be performed with different associations involving cements and resin adhesives. Etch-and-rinse adhesive systems are the oldest of the multi-generation evolution of resin bonding systems. In the 3-step version, they involve acid-etching, priming, and application of a separate adhesive. Acid-etching using phosphoric acid simultaneously etches enamel and dentin. However, etch-and-rinse adhesives produce higher resin-dentin bonds that are more durable than most 1- and 2-step adhesives [10].

Adhesive systems are available in a wide range of commercial products with a different number of bottles and application steps, factors that can also interfere with the strength of the tooth/resin cement bond. Thus, phosphoric acid etching prior to the application of self-etching adhesives clearly demonstrated different bond tendencies between enamel and dentin and was dependent on the adhesive material and tooth substrate [11]. When bonded to dentin, the adhesives with simplified application procedures (one-step self-etch adhesives) still underperform as compared to conventional 3-step adhesives. Two-step self-etch adhesives that provide additional chemical bonding appear to most optimally combine bonding effectiveness with a simplified application protocol [12].

For this reason, the adhesive cementation technique becomes complex, sensitive, and susceptible to operator care. A study evaluating the marginal adaptation of direct resin composites with simulated application errors using dentin adhesives of the third (self-etching primer), fourth (total etching), and fifth generation (one-bottle adhesive) showed that errors resulted in decreased bond strength and reduced percentage of gap-free margins for all the products tested. On the other hand, excessive drying after conditioning exhibited significantly less effect for the third-generation adhesive than for products requiring total etching/wet bonding [13]. In addition, it has been claimed that the interfacial strength and adaptation of self-etching and self-adhesive dual-curing cements are influenced by the cement type, luting pressure, and the interaction between these two factors [14].

Given the aforementioned considerations regarding the effect of marginal adaptation of resin adhesives on the strength of the tooth/restoration bond and the behavior of the adhesive types in different application procedures, in vitro studies on microleakage are essential to predict marginal sealing and durability of the bonding in the long term. A recent study showed that the use of an adhesive-base technique can reduce marginal microleakage, including those in bulk-fill composite restorations [15].

Therefore, the evaluation of microleakage at the bonding interface of different adhesive luting systems associated with water storage simulating the humidity of the oral environment would be important to predict the tooth/restoration marginal integrity. Based on these considerations, the aim of this in vitro study was to evaluate microleakage at the bonding interface that occurred with the conventional 3-step adhesive, self-etching adhesive, and self-adhesive cement techniques after water storage at both 7-day or 6-month periods. The study hypothesis was that different adhesive techniques would promote different microleakage levels in both evaluation times.

2. MATERIALS AND METHODS

2.1. Experimental Protocol

This study was developed using 30 experimental samples and marginal infiltration as an investigated factor. The three-level adhesive cementation technique: CTS+RX–conventional 3-step adhesive (Adapter Scotchbond Multi-purpose, 3M ESPE, St Paul, MN, USA)
+ dual-cure resin cement (RelyX Ultimate, 3M ESPE); SBS+RX—single-bottle self-etching adhesive (Single bond Universal 3M ESPE) + dual-cure resin cement (RelyX Ultimate, 3M ESPE), and SAC—self-adhesive cement (RelyX U200, 3M ESPE). Sample evaluation was performed in two levels: 1) Water storage for 7 days (control) or 6 months at 37.5 °C. Response variable: marginal microleakage in two levels; penetration and quantification of the dye at the adhesive interface.

2.2. Tooth and Dentine Blocks Preparation

Thirty bovine teeth were submitted to manual scraping with a periodontal curette to remove organic debris, polished with Robinson-type brushes (Microdont, Sao Paulo, SP, Brazil) and pumice paste (SS White, Rio de Janeiro, RJ, Brazil) and water. The teeth were sectioned using a metallographic cutter (Isomet 1000, Buehler, LakeBluff, IL, United States) with a diamond disc to obtain dentin blocks with dimensions of 5 mm × 5 mm × 1 mm were made with one side completely in dentin substract. The dentin block was flattened on a metallographic polisher (APL-4, Arotec, Cotia, SP, Brazil) using #600 grit sandpaper (Waterproof; Carborundum Abrasives, Sao Paulo, SP, Brazil), washed in an ultrasonic bath (Unique Ultrasonic Cleaner, Indaiatuba, SP, Brazil) for 10 minutes and stored in distilled water until use.

2.3. Resin Blocks Preparation

Thirty blocks of light-curing composite resin (Z350 XT, 3M ESPE) were made with dimensions of 5 mm × 5 mm × 1 mm using silicone molds. The resin blocks were polished with #600 sandpaper, washed in an ultrasonic bath for 10 minutes, and the bonding surface was blasted with aluminium oxide particles (≤50 μm) with a pressure of 2 bar (30 psi).

2.4. Blocks Cementation

Dentin and resin blocks were randomly separated into three groups of 15 units each and bonded according to the following adhesive cementation techniques.

CTS+RX—conditioning of dentin blocks with 37% phosphoric acid for 15 seconds, washing for the same time, and with water excess removed. The adhesive protocol (Adapter Scotchbond Multi-Purpose) was performed as indicated by the manufacturer for total crown cementation (mix 1 drop of activator with 1 drop of primer), with application to the dentin block and air jet for 5 seconds. The catalyst was applied to the dentin block and resin block previously treated with silane. Dual-cure cement (RelyX Ultimate) was applied to the bonding surface and blocks (resin and dentin) bonded with the aid of static pressure (10N). After removing the excess, the cement was photoactivated for 20 seconds (Ultra-Lume LED 5) on each dentin/resin interface.

SAC—Excess moisture was removed from the dentin and self-adhesive cement (RelyX U200) was applied to the bonding surface. The blocks (resin and dentin) were bonded under static pressure (10N). After removing the excess, the cement was photoactivated for 20 seconds (Ultra-Lume LED 5) on each dentin/resin interface.

All blocks were finished and polished with aluminium oxide discs (Sof-Lex Pop-on; 3M ESPE) in descending abrasiveness order. Afterwards, they were stored in distilled water at 37.5 °C for periods of 7 days (control) or 6 months.

2.5. Microleakage Test

With the exception of the 4 mm wide tooth/resin interface area, the blocks were isolated with two layers of fast-setting cyanoacrylate-based glue (Superbonder; Loc-tite, Itapevi, SP, Brazil) and immersed in a neutral 2% methylene blue solution (Merck, Darmstadt, Germany) for 2 hours. After this time, the blocks were removed from the dye solution, washed in running water, and dried. To remove the dye deposited on the block surface, a light abrasion (0.05 mm) was performed and checked with a digital caliper (Mitutoyo, Tokyo, Japan).

The blocks were sectioned perpendicularly to the cementation margin and the microleakage was visually assessed related to the penetration depth using a stereoscopic magnifying glass (Meiji Techno 2000; Tokyo, Japan) at 40× magnification [16]. The analysis of the amount of infiltrated pigment (μm) was performed with spectrophotometry using a Beckman DU 65 apparatus (Instruments, Fullerton, CA, USA), according to the technique used in previous studies [17, 18]. The data were submitted to two-way ANOVA and Tukey’s test for comparative analysis between groups at p < 0.05 significance.

3. Results

Table I shows the microleakage values that occurred in the different adhesive techniques in relation to the times of 7 days and 6 months of water storage. Conventional 3-step and self-etching techniques did not show statistical differences for microleakage for either the 7-day (p = 0.816) or 6-month (p = 0.997) assessments. The self-adhesive technique showed a higher infiltration value (p < 0.05)

| TABLE I: MEAN AND STANDARD DEVIATION OF THE MICROLEAKAGE (μM) IN THE ADHESIVE TECHNIQUES WITH 7 DAYS OR 6 MONTHS OF WATER STORAGE |
|-----------------|-----------------|-----------------|
| **Time**        | **Conventional 3-step** | **Self-etching** | **Self-adhesive** |
| 7 days          | 18 (25) a, A     | 43 (45) a, A    | 263 (98) b, A    |
| 6 months        | 42 (55) a, A     | 52 (87) a, A    | 441 (226) b, A   |

Note: Means followed by the same letters (lowercase in each row and uppercase in each column) indicate statistical similarity by the Tukey’s test (a < 0.05).
and was statistically different compared to the other treatments. Conventional 3-step, self-etching, and self-adhesive techniques showed values with statistical similarity when the 7-day and 6-month evaluation times were compared.

4. Discussion

This in vitro study verified dye microleakage in different adhesive techniques in relation to the time periods of 7 days or 6 months of water storage. A similarity was found between the conventional 3-step and self-etching techniques for infiltration at both the 7-day or 6-month periods, while the self-adhesive technique showed a higher value. Conventional 3-step, self-etching, and self-adhesive techniques showed no differences between both the evaluation times. Considering these results, the study hypothesis that different adhesive techniques would promote different microleakage levels was confirmed only for the self-adhesive technique in both evaluation times (Table I; Fig. 1).

It has been claimed that the integrity of the marginal seal is a primary factor for the clinical success of adhesive restorations, since dental cements promote different bonding abilities, while resin cement results in a zero percentage of microleakage compared to self-adhesive cementation [3]. The conventional 3-step technique was considered complex, sensitive, and susceptible to operator care, whose deficiencies in the application of the adhesive system can negatively influence the quality of the bonding [13]. On the other hand,简化 procedures should also be considered relevant due to lower technical sensitivity and shorter clinical

It is possible to assume that the different results between studies are due to different sample types and different evaluation times for the baseline (control group). The above-mentioned study evaluated marginal microleakage in Class II cavity restorations. The complex design of such cavities may have influenced the stress/strain ratio during the polymerization of materials based on methacrylates, compromising the quality of the bond six months after water storage and thermocycling. In the current study, the bonding interface was flat and likely more prone to homogeneous stresses in the bonding area of the dentin/cement blocks without thermocycling. Studies with finite elements could clarify these considerations.

On the other hand, the conventional 3-step adhesive cementation and self-etching system showed lower levels of microleakage compared to self-adhesive cementation in both evaluation times (Table I; Fig. 1). This may be due to the better bonding level between the materials, differenting the microleakage caused by the challenges of storage in water for 7-day or 6-month periods. This result could also be explained by the low degradation of the adhesive interface that was not submitted to thermocycling. Moreover, one recent study showed that adhesive-base techniques can reduce marginal microleakage, including those in bulk-fill composite restorations [15].

Contrary to the other adhesive techniques, the self-adhesive procedure showed the highest marginal microleakage levels in both evaluation periods (7 days or 6 months). It is possible to assume that this result would be related to deficient formation of the hybrid layer, dificulting the dentin/resin bonding since this adhesive method does not recommend prior acid etching. In addition, one previous study showed that the interaction of each resin cement with dentin substract resulted in specific bond strength and failure patterns that varied among commercial resin cements evaluated at 24 hours or 30 days. However, even though the self-adhesive cement did not form an authentic hybrid layer, its absence did not prevent a reliable adhesion with the underlying dentin [19].

Although the conventional 3-step and self-etching cementing techniques showed lower levels of microleakage, the conventional 3-step technique was considered complex, sensitive, and susceptible to operator care, whose deficiencies in the application of the adhesive system can negatively influence the quality of the bonding [13]. On the other hand, simplified procedures should also be considered relevant due to lower technical sensitivity and shorter clinical
time. The stability of resin restorations in the oral environment is also highly dependent on the structure of the monomers of composite and adhesive systems. Moreover, the issues related to the microleakage of fluids into the gap and bacteria leaching from the surface of composites are the main causes of failures in adhesive restorations [20].

Photopolymerization is a complex reaction that has several clinical implications due to material composition and it is also influenced by multiple factors including substrate characteristics, operator technique and light-cure unit properties [21]. The degree of conversion of the adhesive can be affected by the functional monomer and depends on the photo-initiator system. Thus, as the bond durability depends also on the strength and degree of conversion of the adhesive, the interaction between the adhesive and photo-initiator system definitely needs to be clinically considered [22]. Nonetheless, different photoactivation sources and increasing light-curing time were not factors influencing the microleakage in restorative procedures with two-step etch-and-rise adhesive system nanofilled and composite resin submitted to thermal and mechanical loading cyclings [23].

In clinical terms, one comparative study showed that occlusal microleakage in cervical composite restorations using conventional and two-step self-etch adhesives in five study groups was dependent on the dental substrate, adhesive material, and technique applied. The Clearfil SE bond/self-etch group showed the highest microleakage in dentin margins compared to the G-Premio bond/selective-etch technique group with the lowest dentin microleakage [24].

In the present study, composite resin blocks bonded to bovine tooth dentin with self-adhesive cement showed the highest mean level of marginal leakage compared to conventional 3-step adhesive or single-bottle self-etching adhesive. Therefore, the investigation’s lack of different photoactivation sources, degree of conversion, and light-curing time are variables that can be considered as limitations of the study.

5. Conclusions

Regardless of study limitation, the following conclusions can be considered: microleakage levels for conventional 3-step or self-etching techniques were similar at the 7-day or 6-month periods. The self-adhesive technique showed a higher microleakage level at the 7-day or 6-month periods compared to other adhesive techniques. There was a statistically significant difference between the evaluation times only for the self-adhesive technique.

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Conflict of Interest

Authors declare that they do not have any conflict of interest.

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