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Full Length Research Paper

The effect of storage and type of adhesive resin on microleakage of enamel margins in class V composite restorations

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This study compared microleakage of enamel margins in class V cavities restored with two types of adhesives at three time intervals. A total of 120 bovine incisors were randomly divided into two groups (groups 1 and 2) according to the type of the adhesive used (dentin and enamel adhesives, respectively). Then, each group was divided into three subgroups (n = 20) (subgroups 1 to 3: evaluation of microleakage at 24 h, 6 months and 12 months intervals after restoration, respectively). Subsequent to restoration and immersion in fuschin, the teeth were sectioned and microleakage was evaluated. Kruskal-Wallis and Mann-Whitney U tests were used for comparison of microleakage of the three subgroups in each group and for two-by-two comparisons, respectively. Mann-Whitney U test was used for comparison of microleakage between enamel and dentin adhesives at each time interval. There were significant differences in the microleakage between the three time intervals in both adhesives ($p < 0.001$). The differences in microleakage between the two adhesives were significant at 12 month interval ($P = 0.02$), whereas there were no significant differences in the microleakage at other intervals between the two adhesives ($p > 0.05$). Dentin adhesive showed a better durability of the bond to enamel when compared to enamel adhesive subsequent to 12 months of storage in water.

Key words: Enamel adhesive resin, dentin adhesive resin, microleakage, water storage.

INTRODUCTION

In recent years, adhesive techniques which preserve tooth structures have gained popularity over techniques which provide mechanical retention for restorative materials. The principal purpose of bonding to tooth structures is to produce a durable and appropriate bond between the restorative material and tooth structures (Perdigao and Swift, 2006). Several adhesive resins have been introduced to improve the bond strength, facilitate and simplify bonding procedure steps (Van Meerbeek et al., 2006). Bond strength of adhesive restorations is of utmost importance for their clinical success (De Munck et al., 2005).

In vitro studies have demonstrated that the majority of adhesive resins have high bond strength values; however, after a year of storage in water, they exhibit a significant decrease in bond strength (Perdigao and Swift, 2006; De Munck et al., 2005). Some researchers have attributed the decrease in bond strength values to the penetration of water into the resin-tooth structure interface and the hydrolysis of the bond. Hydrolysis might result from degradation of resin or tooth structure collagen (De Munck et al., 2005; Jaffer et al., 2002; Finer and Santerre, 2004). It has been reported that the hydrolytic degradation of the bond can influence the bonding efficacy (bond strength and marginal seal) of the bond in the long run (Hashimoto et al., 2002, 2003, 2007). Decreased bond strength and increased

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Table 1. Previous studies conducted on enamel-resin bond durability.

Type of adhesive	Name of resin	Investigated parameter	Duration	Reference
Dentin-bonding adhesives (self-etch, and etch and rinse)	Single Bond, Prime & Bond NT, Clearfil SE Bond, Resulcin Aquaprime, NRC/Prime & Bond NT, Etch & Prime 3.0, Adper Prompt-L-Pop	Enamel-resin bond durability	24 h, 6 months and 1 year	Osorio et al. (2009)
Dentin-bonding adhesives (self-etch)	Clearfil S3 Bond, Adper Prompt L-Pop, iBond, Clearfil SE Bond	Enamel-resin bond durability	24 h, 6 months and 1 year	Reis et al. (2009)
Dentin-bonding adhesives (self-etch, and etch and rinse)	Clearfil SE Bond, Optibond Solo Plus Self-Etch Primer, AdheSE, Tyrian Self Priming Etchant + One Step Plus, Single Bond, Scotch Bond Multi Purpose	Enamel-resin bond durability	24 h and 1 year	Loguercio et al. (2008)
Dentin-bonding adhesives (self-etch)	OBF-2, i Bond, Adper Prompt L-Pop	Enamel-resin bond durability	24 h and 1 year	Foxton et al. (2008)
Dentin-bonding adhesives (self-etch, and etch and rinse)	Imperva Fluoro Bond, Clearfil Liner Bond II, Mac Bond II, One Step, OptiBond Solo, Prime & Bond 2.0, Single Bond	Enamel-resin bond durability	24 h followed by thermal cycling between 5 and 60° C for 3,000, 10,000, and 30,000 cycles	Miyazaki et al. (2000)

microleakage result in tooth hypersensitivity, marginal discoloration, recurrent caries and pulp irritation (Perdigao and Swift, 2006; Van Meerbeek et al., 2006). At present, two kinds of adhesive resins are available for bonding to tooth structures. They include enamel-bonding and dentin-bonding adhesive systems (Perdigao and Swift, 2006; Van Meerbeek et al., 2006).

Enamel-bonding systems are hydrophobic and can only be applied to the enamel; however, dentin-bonding systems are hydrophilic and can be applied to both the enamel and dentin. As a result of the hydrophilic nature of dentin surfaces, only hydrophilic bonding systems are capable of producing an appropriate bond with dentin. Nevertheless, such systems are more susceptible to hydrolysis (Van Meerbeek et al., 2006; Powers and Sakaguchi, 2006). Previous studies conducted on enamel-resin bond durability using dentin-bonding adhesives are summarized in Table 1 (Osorio et al., 2009; Reis et al., 2009; Loguercio et al., 2008; Foxton et al., 2008; Miyazaki et al., 2000).

Since no studies have to date evaluated enamel margin microleakage of enamel-bonding adhesive systems in the long run, the aim of this *in vitro* study was to compare enamel margin microleakage in class V cavities of bovine teeth restored with two adhesive resins (enamel- and dentin-bonding adhesives) at 24 h, 6 months and 12 months intervals post-operatively.

MATERIALS AND METHODS

In this *in vitro* study, 120 sound bovine (Borges et al., 2007; Nakamichi et al., 1983) incisor teeth were used. The teeth were free of any cracks, fractures, abrasions and structural defects as evidenced by visual evaluation and examination under a stereomicroscope (Nikon, Tokyo, Japan). The teeth were stored in 0.5% chloramines T solution until used for the purpose of the study. Before the study, the teeth were cleansed of any calculi and cleaned and polished with pumice and rubber cups. The samples were randomly divided into two groups of 60 based on the type of the adhesive resin used: enamel and dentin-bonding adhesive resins. Table 2 summarizes the particulars of the resins used. In each group, the teeth were randomly divided into three subgroups of 20: subgroup 1, evaluation of microleakage 24 h after restoration; subgroups 2 and 3, evaluation of microleakage at 6 months and 12 months post-operative intervals, respectively. In all the samples, class V cavities were prepared on the buccal surface; the cavities measured 3 × 3 occluso-gingivally and mesio-distally and were 2 mm deep (Borges et al., 2007). Both occlusal and gingival margins of the cavities were placed in enamel with the gingival margin 2 mm above the CEJ (cemento-enamel junction). Cavity dimensions were demarcated on each tooth before cavity preparation using a template; cavity depth was measured with a periodontal probe. The cavities were prepared with 01 fissure diamond burs (Diatech Dental AG, Switzerland Dental Instruments, CH-9435 Heerbrugg) in a high-speed handpiece under air and water cooling. A new bur was used after 5 cavity preparation procedures (Amaral et al., 2004). All the cavity margins were butt-jointed without any bevels (Santini et al., 2004). Subsequent to etching, the cavity walls with 37% phosphoric acid (3M ESPE, St. Paul, MN, USA) according to the manufacturer's instructions, and Adper Single Bond adhesive

Table 2. Chemical composition of the adhesive resins used in the study.

Adhesive resin type	Name	Manufacturer	Composition
Dentin adhesive resin	Adper Single Bond	3M ESPE, St. Paul, MN, USA	Bis-GMA[1], HEMA[2], ethanol, water, initiator (camphoroquinone), dimethacrylates, polyalkenoic acid copolymer
Enamel adhesive resin	Margin Bond	Coltène/Whaledent AG, Switzerland	Bis-GMA[1], TEGDMA[3]

[1]Bisphenol-glycidyl methacrylate; [2]hydroxyethyl methacrylate; [3]triethylene glycol dimethacrylate.

Table 3. Study groups and subgroups.

Group	Subgroup
Group 1 (Dentin adhesive resin)	Subgroup A: evaluation of microleakage 24 h subsequent to restoration Subgroup B: evaluation of microleakage 6 months subsequent to restoration Subgroup C: evaluation of microleakage 12 months subsequent to restoration
Group 2 (Enamel adhesive resin)	Subgroup D: evaluation of microleakage 24 h subsequent to restoration Subgroup E: evaluation of microleakage 6 months subsequent to restoration Subgroup F: evaluation of microleakage 12 months subsequent to restoration

resin (3M ESPE, St. Paul, MN, USA) were used in the three subgroups of group 1 (A, B and C) and Margin Bond (Coltene Whaledent, Switzerland) adhesive resin was used in the three subgroups of group 2 (D, E and F); both resins were used according to the manufacturer's instructions. Astralis 7 light-curing unit (Ivoclar Vivadent, FL-9494 Schaan, Liechtenstein) was used to light-cure the resins; the probe was 8 mm in diameter and the light intensity was 400 mW/cm^2. The tip was placed perpendicular to the surface and curing continued for 20 s according to the manufacturer's instructions. To restore cavities in all the groups, Valux™ Plus (3M ESPE, St. Paul, MN, USA) composite was used with incremental technique and each layer was cured for 40 s using Astralis 7 light-curing unit at a light intensity of 400 mW/cm^2.

After restoration, the samples were polished with diamond polishing burs (Diamant Gmbh, D & Z, Goerzallee 307, 14167 Berlin, Germany) and polishing disks (Sof-Lex™, 3M ESPE, St. Paul, MN, USA). To simulate oral cavity conditions, a thermocycling procedure was undertaken, which consisted of 500 cycles at 5 ± 2°C / 55 ± 2°C with a dwell time of 30 s and transfer time of 10 s. Then the samples in subgroups A and D, B and E, and C and F were incubated for 24 h, 6 months and 12 months, respectively (Table 3), in distilled water at 37°C (Crim and Chapman, 1994; Okuda et al., 2002). After each group's specific interval, the teeth were retrieved from distilled water in the incubators and dried. Then the teeth were covered with two layers of nail varnish up to 1 mm from the margins of restorations. The apex of each tooth was covered with sticky wax. The teeth were subsequently immersed in 2% basic fuschin solution (Hashimoto et al., 2000). The teeth were then divided into two halves in a bucco-lingual direction using a diamond disk (Diamant Gmbh, D & Z, Goerzallee 307, 14167 Berlin, Germany). The samples were evaluated under a stereomicroscope (Nikon, Japan) at a magnification of ×16 by two examiners so that dye penetration at gingival or occlusal margins could be classified as follows (Borges et al., 2007): 0: No dye penetration; I: Dye penetration along the gingival or occlusal wall without axial wall involvement; II: Dye penetration along the gingival or occlusal wall with axial wall involvement; III: Dye penetration beyond the gingival, occlusal or axial wall toward the pulp.

After evaluation of the depth of the dye's penetration at the two margins, the score of the margin (gingival or occlusal) with the greatest dye penetration was recorded as the microleakage score of that specimen.

The non-parametric Kruskal-Wallis test was used to compare microleakage at the three intervals and between the two adhesive resins. Mann-Whitney U test was used for the two-by-two comparison of the groups. This latter test was also used to compare microleakage between the three time intervals and between the two adhesive resins. Statistical significance was defined at $p < 0.05$.

RESULTS

The frequency of microleakage scores in the subgroups in this study are demonstrated in Table 4. Kruskal-Wallis test demonstrated statistically significant differences in microleakage at the three time intervals between the two adhesive resins ($p < 0.001$). Two-by-two comparison of the groups demonstrated statistically significant differences in microleakage between 24 h and 6 months intervals, and between 24 h and 12 months intervals ($p \leq 0.001$); however, there were no significant differences in microleakage between the 6 months and 12 months intervals ($p > 0.05$).

Furthermore, there were no significant differences in microleakage of the two adhesive resins at 24 h and 6 months intervals ($p = 0.50$ and $p = 0.31$, respectively); however, there were significant differences in microleakage between the two adhesive resins at 12 months intervals ($p = 0.02$). Calculated values and results using Kruskal-Wallis and Man-Whitney U tests are presented in Tables 5 and 6. Microleakage scores of the selected samples are represented in Figure 1.

Table 4. Microleakage scores in the subgroups.

Subgroup	Microleakage score				
	0	I	II	III	Total
A	6	9	5	0	20
B	0	6	8	6	20
C	1	3	14	2	20
D	6	6	7	1	20
E	0	4	7	9	20
F	0	0	14	6	20

Table 5. Kruskal-Wallis and Man-Whitney U test results for comparison of microleakage at the three intervals in each adhesive.

Type of adhesive	Type of statistical test	Comparison	Test statistics
Dentin adhesive	Kruskal-Wallis	Three time intervals	$X^2 = 16.59$, df = 2, p < 0.001
	Mann-Whitney U	24 h and 6 months	U = 77.00, p < 0.001
	Mann-Whitney U	24 h and 12 months	U = 80.50, p < 0.001
	Mann-Whitney U	6 months and 12 month	U = 183.00, P = 0.61
Enamel adhesive	Kruskal-Wallis	Three time intervals	$X^2 = 18.16$, df = 2, p < 0.001
	Mann-Whitney U	24 h and 6 months	U = 80.00, P = 0.001
	Mann-Whitney U	24 h and 12 months	U = 66.50, p < 0.001
	Mann-Whitney U	6 month and 12 months	U = 198.00, P = 0.95

Table 6. Man-Whitney U test results for comparison of microleakage between two adhesives in each time interval.

Time interval	Type of statistical test	Comparison	Test statistics
24 h	Mann-Whitney U	Dentin and enamel adhesives	U = 176.50, P = 0.50
6 months	Mann-Whitney U	Dentin and enamel adhesives	U = 165.00, P = 0.31
12 months	Mann-Whitney U	Dentin and enamel adhesives	U = 132.00, P = 0.02

DISCUSSION

Longevity of the bond is an important factor in the clinical efficacy of adhesive restorations. In this study, the teeth restored with both the adhesive resins exhibited increased microleakage at enamel margins with time. In the same context, various *in vitro* studies have shown that long-term storage in water results in defects in bonded interfaces (Amaral et al., 2004; Koshiro et al., 2004; Malacarne et al., 2006; Armstrong et al., 2001; De Munck et al., 2003). In addition, it has been reported that sealing ability of total-etch and self-etch adhesive resins at enamel margins decreases with time (Malacarne et al., 2006; Osorio et al., 2009), which might be attributed to the absorption of water into the polymer networks with time and the subsequent hydrolytic degradation of adhesive resins.

The results of this study showed that the differences between the microleakage of Adper Single Bond and Margin Bond adhesive resins at 24 h and 6 months intervals were not statistically significant; however, at 12 months interval, Adper Single Bond group demonstrated significantly less microleakage than the Margin Bond group. The lack of significant differences between the two adhesives at 24 h and 6 months intervals might be attributed to the insufficient time necessary for observable changes in the polymer network and its degradation to take place. The lower microleakage of Adper Single Bond adhesive at the 12 months interval might be attributed to the chemical composition of the components and differences in the chemical behaviors of the two adhesive resins in the aqueous solutions in the long run. The presence of dimethacrylates in the chemical composition of Adper Single Bond (Table 1) resulted in strong cross-linkings; therefore, the resultant polymer was less soluble in water with a lower rate of hydrolysis (Malacarne et al., 2006; Ferracane, 2006). On the other hand, there is a polyalkenoic acid prefabricated polymer (PAC) or its co-polymers (Table 2) in the chemical composition of Adper Single Bond, the esters of which are usually alkenoic acid. This chemical agent results in the light-activated polymerization of bisphenol-

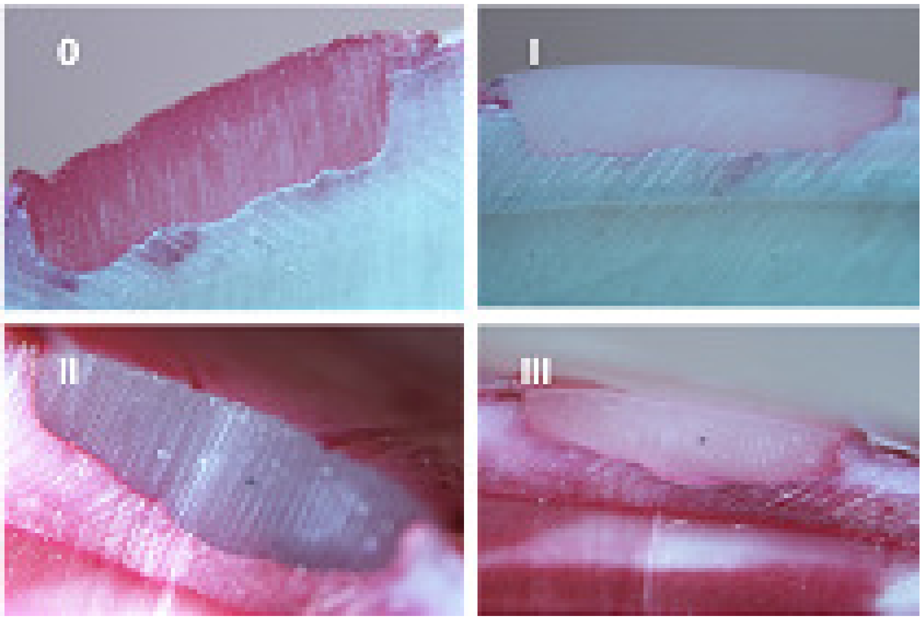

Figure 1. Microleakage scores (0 to III) in the selected samples.

glycidyl methacrylate (Bis-GMA) monomers, hydroxyethyl methacrylate (HEMA) and dimethacrylates inside the polymer bed of the prefabricated polyalkenoic acid, leading to the formation of intertwined polymer networks called interpenetrated networks. These networks improve polymer strength (Nakabayashi, 2008), decrease water permeability (Abbasi et al., 2001) and increase longevity and resistance of the polymer to hydrolytic degradation (Kim et al., 2005).

On the other hand, Margin Bond contains Bis-GMA and triethylene glycol dimethacrylate (TEGDMA) monomers (Table 2). Both monomers are dimethacrylates and produce polymer networks with cross-linkings. They produce more pores and cavities for water penetration and are more susceptible to hydrolysis as compared to linear polymers (Malacarne et al., 2006; Ferracane, 2006; Rivera-Torres and Vera-Graziano, 2008). It seems that the presence of more pores and cavities in the polymer structure of Margin Bond as compared to Adper Single Bond had a role in its wear behavior in water. In addition, no intertwined polymer networks were produced in the polymer structure of Margin Bond; therefore, the presence of a protective effect against hydrolysis was not possible.

In this study, it was not possible to evaluate the solubility parameters of the adhesive resins used because manufacturers do not disclose the exact chemical composition and quantity of each component, which is necessary for such evaluations. Since the rate and polymer hydrolysis percentage are under the influence of pH and temperature, in addition to the influence of polymer nature and the percentage of the constituent polymers, it is suggested that future microleakage studies be carried out *in vivo*. It is also suggested that the composite-enamel interface should be evaluated by electron microscopy.

According to the results of this study, Adper Single Bond adhesive resin demonstrated a better durability of bonding to enamel when compared to Margin Bond adhesive resin after a year of storage in water.

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