

The Impact of Water Storage on Microleakage of Two Generations of Dentin Bonding Agents: An In-Vitro Study

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Abstract

Objectives: The latest generation of bonding agents is increasingly embraced and well-suited to fulfilling the requirements for durable restorations. This study aimed to assess microleakage levels of three self-etch adhesives (Clearfil SE (SE), Protect (PB), and Tri-S (TS) Bonds) in composite restorations at both occlusal and cervical borders, while considering the impact of water storage over different time intervals.

Methods: Standard Class V cavities were prepared on buccal surfaces of 84 human premolars at the level of cemento-enamel junction. The samples were randomly assigned to six groups (N=14). The corresponding bonding procedure in each group was completed, and the cavities were filled using Clearfil AP-X (Kuraray). Following thermocycling, the teeth were stored in deionized water within an incubator for 24-hour and 6-month intervals. Statistical analysis of microleakage data was conducted utilizing Kruskal-Wallis and Mann-Whitney U tests at $p < 0.05$.

Results: Following a 24-hour interval, the level of microleakage at the occlusal surface was greater than the gingival surface for SE. No statistically significant differences were observed in microleakage at the occlusal and cervical borders after 24h of water storage for the PB and TS groups ($P=0.51, 0.16$). None of the SE and TS group samples showed leakage at the occlusal or cervical margins following a 6-month interval.

Conclusion: Self-etching bonding agents indicated increased occlusal microleakage compared to the cervical margin microleakage.

Keywords: Dentin-Bonding Agents; Dental Leakage; Dental Restoration Failure; Dental Bonding

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Introduction

Recent advancements in adhesive technology have played a crucial role in the rapid progress of biomimetic dentistry.^{1,2} To ensure an effective and durable restoration, it is critical to establish an effective bond between the two adhesive interfaces and the tooth substrate, allowing for the attainment of an ideal seal. Polymerization shrinkage is a common issue with light-cured composite resins, leading to shrinkage stress that can impact marginal integrity and cause problems like marginal leakage, debonding, and postoperative tooth hypersensitivity. The penetration of bacteria and oral fluids into the gap created due to polymerization shrinkage at the tooth-restoration interface causes a marginal color change in restoration, ultimately leading to secondary caries in long term.^{2,3} Bonding agents have evolved to provide improved long-term durability, with self-etch bonding agents being favored due to advantages such as lower technical sensitivity, time management, and the chemical adhesion possibility in dentin.⁴

The categorization of self-etch bonding agents relies on the number of stages utilized in the process. Self-etch adhesives were introduced to the market, incorporating a 10-methacryloxydecyl dihydrogen phosphate (10-MDP) acidic monomer. In the 6th generation, the first generation of self-etch adhesives, the acidic monomer and primer were presented in one bottle, and the adhesive in another. In the 7th generation, all three components were placed in a single bottle, making it easier and more convenient for application. This system is particularly beneficial for

patients with difficulty sitting in the dental chair for extended periods, such as children or elderly individuals.⁴⁻⁶ A high-quality layer between dentin and restoration is formed by creating a hybrid layer with these adhesives, which occurs due to dentin demineralization and the impregnation of adhesives in demineralized areas.^{4,7} To ensure an effective adhesive restoration treatment, it is crucial to demineralize the dentin substrate and ensure proper penetration of the primer for effective resin impregnation. This forms a strong hybrid layer, essential for long-term durability and high bond strength. Achieving sufficient mechanical strength in the cured resin adhesive is paramount.⁸⁻¹⁰

The resin matrix inherently causes shrinkage during polymerization, which can lead to residual stresses in adhesives and create a gap between the two interfaces, depending on the degree of the shrinkage.^{1-3,11-13} The handling properties of methacrylate monomers, commonly used as the resin matrix in dental adhesives, may be affected by their molecular weight and viscosity, potentially causing additional polymerization shrinkage. The main factor contributing to volumetric shrinkage during the polymerization of methacrylates is the transformation of double bonds into single bonds.¹¹ Due to the higher pH of acidic monomers, the smear layer is not completely removed and is referred to as modified in self-etch bonding systems. Due to the presence of bacteria and their residues in this layer, the antibacterial properties in self-etch bonding systems can help with the long-term durability of restorations.¹⁴ Using methacryloxydecyl pyridinium bromide (MDPB) in

resin-based materials for dental caries treatment has shown promising results in enhancing prognosis by incorporating antibacterial properties. MDPB monomer combines a quaternary ammonium antibacterial agent with a methacryloyl group, demonstrating potent antibacterial effects against oral streptococci. Evidence shows that including MDPB successfully equips dentin bonding systems with antibacterial capabilities pre- and post-curing.¹¹⁻¹⁷

The durability of adhesive systems is crucial for the extended effectiveness of dental restorations. Since bonding agents typically deteriorate over time, examining the gingival and cervical microleakage of different self-etch bonds was implemented in this study. Specifically, two 6th-generation self-etch bonds containing 10-MDP and MDPB acidic monomers were compared with the 7th-generation bond from the same brand. This study aimed to assess the extent of microleakage in Class V composite restorations at their occlusal and gingival borders following 24-hour and 6-month intervals of water storage using three different adhesives.

Methods

Eighty-four human premolars, extracted for orthodontic reasons, were collected and stored in water. Class V cavities were then prepared on the buccal surfaces using diamond fissure burs (size 008). The preparations were made at the level of cemento-enamel junction, with diameters of 2mm, 2mm, and 3mm, and depths and heights of 2mm, 2mm, and 3mm, respectively. The upper border of the cavity was positioned at the enamel, while the lower border was on the cementum. The sample size was determined to achieve a statistical power of 80%. The specimens were randomly assigned to three groups based on the adhesive type (Clearfil SE (SE), Protect (PB), and Tri-S (TS) Bonds), and each group was further divided into two subgroups based on time intervals (Totally six groups of 14 samples each; I to VI). The bonding agents' compositions are presented in Table 1.

The dentin bonding agents were applied following the instructions provided by the manufacturer. In SE groups I and IV, a two-step adhesive was utilized, with the primer being applied (20s), followed by the bonding agent, and then light curing (10s). A two-step self-etch adhesive was used in the PB groups II and V. The cavity surface was first treated with the primer (20s), followed by air blasting. Then, the bonding agent was applied, air blasted, and cured (10s). On the other hand, in the TS groups III and VI, a one-step self-etch adhesive bonding agent was applied to the cavity surfaces (20s), followed by air blasting and curing (10s).

After bonding agent application, the cavities underwent restoration incrementally using the AP-X composite resin (Kuraray Medical Inc., Tokyo, Japan). Each layer was subjected to a 30-second curing period. The restorations were finished using carbide burs and polished with a polishing disk. To evaluate microleakage, the teeth were placed in distilled water at a temperature of 37°C. After storage, the specimens were thermocycled for 500 cycles at 5°C and 55°C before testing. Each water bath had a dwell time of 10s, with a transfer time of 5s between each bath (Dorsa Iran).

Table 1- Name, composition and manufacturer of the bonding materials

Adhesive	Composition
Clearfil SE Bond (Kuraray, Medical Inc., Tokyo, Japan)	MDP HEMA Hydrophilic dimethacrylate dl-Camphorquinone N,N-Diethanol-p-toluidine Water Bis-GMA Silanated colloidal silica
	MDP HEMA MDPB Hydrophilic dimethacrylate Water Bis-GMA Hydrophobic dimethacrylate N,N-Diethanol-p-toluidine Silanated colloidal silica Surface treated sodium fluoride
Clearfil Tri-S Bond (Kuraray, Medical Inc., Tokyo, Japan)	MDP Bis-GMA HEMA Hydrophobic dimethacrylate dl-Camphorquinone Ethyl alcohol Water Silanated colloidal silica

Composition as provided by respective manufacturer:

MDP: 10-Methacryloyloxydecyl dihydrogen phosphate;

HEMA: 2-Hydroxyethyl methacrylate;

Bis-GMA: Bisphenol-glycidyl methacrylate

MDPB: 10-Methacryloyloxydodecylpyridinium bromide

The roots of specimens were covered with Clearfil AP-X flow composite. A nail varnish was administered to all root surfaces, excluding a 1mm region near the borders of the Class V composite restoration. The specimens were subsequently encased in acrylic mounts, while the root apices were protected with wax. Additionally, the areas, excluding the restorations and a 1mm perimeter from the edges, were covered with dual coatings of nail varnish. Groups I through III were immersed in distilled water for 24h, whereas groups IV, V, and VI were incubated at 37°C for six months. All samples were subjected to a 0.5% basic fuschin dye solution for 24 hours after the storage period. The teeth were then washed and dried before

being longitudinally sectioned using a diamond disc in a buccolingual direction (Isomet, Buehler, Ltd., Lake Bluff, IL, USA). Microleakage was evaluated at the specimens' margins using a stereo microscope (Olympus-Japan) at a magnification of $\times 40$ by two blind independent assessors. The dye penetration score assessment was carried out according to Table 2.

Score of dye penetration	Description
0	No dye penetration
1	Dye penetration up to 1/3 of the distance between the margin and axial wall
2	Dye penetration up to 2/3 of the distance between the margin and axial wall
3	Dye penetration along the axial wall

Statistical analysis

The analysis was conducted using SPSS software (version 15.0, SPSS Inc., Chicago, USA). In cases of non-normal distribution of data, the Kruskal-Wallis test was employed. Specifically, a non-parametric Kruskal-Wallis test was applied to assess the leakage across three sets.

Furthermore, a pairwise comparison of microleakage was carried out using the Mann-Whitney U test. The significance level for all tests was set at 0.05.

Results

The samples from the SE group did not show any evidence of leakage at the occlusal or gingival margins following a 6-month storage period. The analysis conducted with the PB bond agent, revealed no significant differences between the occlusal and cervical margins ($p=0.06$). Additionally, there was no evidence of occlusal and cervical microleakage within the TS bonding adhesive, as the score was 0.

During the 24-hour storage period, it was observed that in the SE group, there was a significant difference in microleakage between the occlusal and cervical borders (Figure 1). The microleakage level, particularly in enamel margins, exhibited a significantly higher value than the gingival border in dentin ($p<0.05$). The occlusal and cervical microleakage results of three self-etch bonding agents at both the 24-hour and 6-month time points are presented in Table 3.

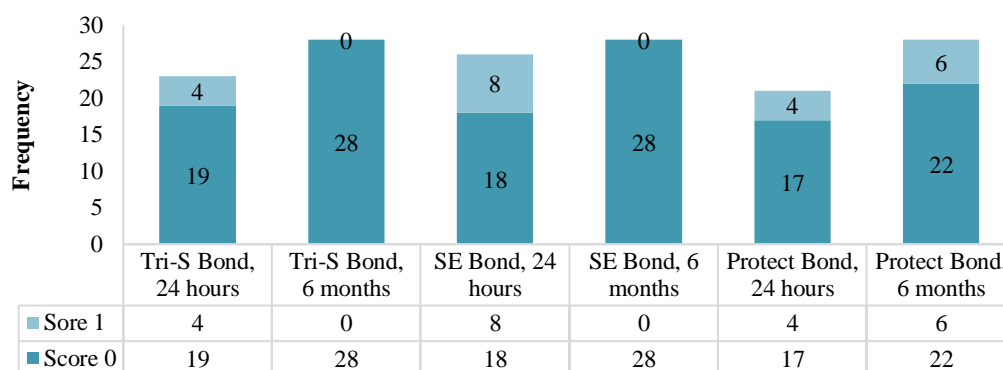


Figure 1: Comparison of microleakage of occlusal and gingival surfaces after 24 hours and 6 months

Table 3- Occlusal and cervical microleakage of three self-etch bonding agents after 24 hours and 6 months

Groups	Duration	No. of specimens	Dentin bonding agent	Mean score	Chi-square	P-value
Occlusal micro leakage	24-h	14	Tri-S Bond	21.79	0.023	0.99
		14	SE Bond	21.14		
		14	Protect Bond	21.57		
	6-m	14	Tri-S Bond	18.5		
		14	SE Bond	18.5		
		14	Protect Bond	27.50		
Cervical micro leakage	24-h	14	Tri-S Bond	20.79	1.319	0.52
		14	SE Bond	20.14		
		14	Protect Bond	23.57		
	6-m	14	Tri-S Bond	21.50		
		14	SE Bond	21.50		
		14	Protect Bond	21.50		

As shown in Figure 1, the findings from the 6-month water storage of samples indicated that there were no

instances of leakage at occlusal and gingival borders in the SE and TS groups. Conversely, the Mann-Whitney U

test conducted on the PB group did not show any significant differences between the margins ($p=0.06$).

A significant variance in statistical terms was noted at the occlusal surface for the SE bond between the 24-hour and 6-month evaluations ($p<0.009$). Similarly, the Mann-Whitney U test for PB demonstrated statistically significant differences at the gingival margin between the 24-hour and 6-month ($p<0.03$). Additionally, the Kruskal-Wallis test conducted in the TS group revealed no statistically significant variances at the gingival borders, indicating that the storage time did not impact the microleakage at the gingival surface. This pattern was also observed for the SE bond.

Discussion

Self-etch adhesives have become widely popular over the years because of their user-friendly nature, simplification of tasks, improved time management, and desirable outcomes.¹⁸⁻²¹ This investigation focused on three self-etch bonding agents—SE, PB, and TS—all of which were manufactured by Kurary, Japan. The degree of microleakage at the enamel and dentin substrate of C1 V cavities was evaluated following 24-hour and 6-month intervals of water storage.

The SE bond (6th generation) is a mild two-bottle adhesive. The functional monomer in the SE primer is 10MDP, which enhances the bonding capability to the tooth structure. 10MDP has the highest interaction with hydroxyapatite and forms the most stable bond with calcium.²²⁻²⁵ Studies indicate that the absence of phosphoric acid in the etching process leads to increased levels of microleakage at the enamel margins.²⁶⁻²⁹ The present findings indicated that following 24h of water storage, there was a significantly higher degree of enamel microleakage compared to the dentin in the SE group. This significant difference demonstrated the importance of considering the substrate when using SE adhesives. However, no level of microleakage was detected in a 6-month interval. Although there was a higher level of leakage at the occlusal surface compared to the cervical samples, there were no statistically significant differences in microleakage between the two borders.

SE bond has a pH of 2, potentially accounting for the increased microleakage at the occlusal compared to the gingival margin.³⁰ The reason for this is that the adhesive is a weak self-etch bonding that cannot fully etch enamel for an optimal bond to form. However, 10-MDP present in this adhesive can form a chemical bond with dentin, ultimately improving the bond's strength as time progresses.^{21,31-33} This chemical's bonding to calcium helps offset the micro-mechanical bond degradation due to hydrolytic activity, giving this system a competitive

edge.

Numerous studies have demonstrated the outstanding performance of SE bond, establishing it as one of the most reliable self-etch adhesives available.^{3,26} Despite the formation of the extremely thin hybrid layer (1 micrometer) and the blockage of the dentinal tubule orifice by a smear plug, which is partly penetrated with resin, there is diminished production of resin tags when using the system.³³⁻³⁶ The hybrid layer may be thin, but research has demonstrated that the bond could reach exceptional dentin bond strength, sometimes surpassing what is achieved with total-etch adhesives.^{8,37-39}

The research carried out by Peumans M. et al. (2007) demonstrated that patients did not encounter any sensitivity or problems following two years of using the bonding agents. Apart from exhibiting positive outcomes in clinical trials, these bonding agents have also shown promising results in laboratory studies.³

Failure to completely remove the smear layer in self-etch generations may result in the persistence of microorganisms within the smear layer. Therefore, the use of adhesives with antibacterial properties can be highly beneficial. Not only can these adhesives destroy bacteria, but they can also improve bonding efficiency and the overall success of the restoration.^{40,41} PB bond is a two-stage self-etch adhesive that includes fluoride. The primer in this adhesive includes MDP and MDPB and has a pH of 2.

The anti-bacterial properties of self-etch adhesives are influenced by various factors, with the acidity of the self-etch primer playing a crucial role in their antibacterial effects.⁴¹

Imazato S. et al. (1998) have reported the potent antibacterial properties of unpolymerized MDPB. By incorporating an acidic monomer into primers, the pH can be reduced sufficiently to diminish bacterial activity. The results of the research reported by Ozer F. et al. (2003) indicate that the bactericidal properties of MDPB monomer in primers are superior to those of other self-etch adhesives.^{12-17,40,41} MDP found in PB primer contributes to its anti-bacterial properties. In contrast, the adhesive component in the second bottle does not display any anti-bacterial effects in the Agar Well Technique.⁴¹

The findings of the current study indicated a higher level of leakage observed at the occlusal border in contrast to the gingival one, although it was not statistically significant. Furthermore, the microleakage observed after 24h exceeded that seen at the 6-month mark, suggesting a reduction over time. This could be attributed to changes in the monomer in PB, which may have antibacterial effects. These changes, along with other formulation changes, could negatively affect the short-term bonding capacity. It is worth noting that MDPB does not contain

acidic groups, which may affect the initial interaction between the bonding agent and tooth substrate. Nevertheless, once the chemical reaction between MDP and enamel reaches completion over time, the impact is compensated. In this research, the improved seal and reduced microleakage of the bonding agent may be attributed to the ongoing chemical reaction within the composite after 24 hours.^{42,43}

Due to a decrease in the number of procedural stages required during the adhesion and improved overall efficiency, a variety of innovative self-etch bonding agents have been introduced to the market.⁶ TS is a single-step self-etch bonding agent that has shown higher microleakage at the occlusal margin in comparison to the cervical one after 24 hours of water storage. Interestingly, after six months, the leakage at the occlusal margin decreased, while the time elapsed had no impact on the amount of microleakage observed at the cervical margin. The increased microleakage observed at the occlusal surface may be attributed to the single-step use of the bonding agent. This method involves using a bonding solution containing water, alcohol, and acetone all in one, which should ideally be evaporated by air spray before the curing process is finished. However, if any solvent residue remains and fails to evaporate completely, it could adversely affect the polymerization reaction. Consequently, the long-term efficacy of the bonding agent may be compromised due to the hydrolysis of its components.^{44,45}

Studies have indicated that single-step self-etch adhesives are composed of more ionic and hydrophilic resin monomers, potentially leading to water retention at the tooth-resin interface post-curing, thereby compromising the restoration seal. Consequently, despite its ease of use

and quick application, the TS bond has not demonstrated satisfactory long-term performance, necessitating further clinical and laboratory investigations.⁴⁶ It is still unclear which factor has the most significant impact on dentin bonding agents. Despite its ease and speed of use, the TS bond has not demonstrated satisfactory long-term performance, necessitating further clinical and laboratory investigations.

Conclusion

Self-etching bonding agents exhibited increased microleakage at the occlusal margin compared to the cervical margin. None of the most recent generations of adhesive resin bonding systems can produce completely sealed margins in Class V composite restorations.

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Data Availability Statement: The research data associated with a paper is available (Data available on request from the authors).

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