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Effect of the Amount of Hydroxyethylmethacrylate in an Experimental Dentin Bonding System on the Degree of Conversion and Microleakage of Class V Composite Restorations

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Abstract

Background: Experimental dentin bonding systems containing nanoclay fillers have shown high microleakage, which may be due to the high concentration of hydroxyethylmethacrylate (HEMA) in their composition.
Objectives: This study sought to assess the effect of using different concentrations of HEMA in an experimental dentin bonding system containing PAA-g-nanoclay on the degree of conversion and microleakage of class V composite restorations.
Methods: This in vitro experimental study was conducted on 60 class V cavities prepared in the buccal and/or lingual surfaces of sound-extracted premolar teeth. The cavities were restored using an experimental dentin bonding agent containing PAA-g-nanoclay and a light-cure composite in three groups (n = 20) with a HEMA concentration of 15% (group 1), 20% (group 2), and 30% (group 3) in the adhesive. After thermocycling, microleakage was assessed at the occlusal and gingival margins of the restorations using the dye penetration method. The degree of conversion of the dentin bonding agent was calculated using Fourier transform infrared spectroscopy. The data was analyzed using SPSS version 16 and the Kruskal-Wallis, Fisher's exact, Wilcoxon, and Mann-Whitney tests ($\alpha = 0.05$).
Results: Microleakage at the occlusal and gingival margins was not significantly different between the three groups ($P > 0.05$), but the difference in microleakage between the occlusal and gingival margins was significant within each group ($P < 0.001$). The three groups were not significantly different in terms of degree of conversion ($P > 0.05$).
Conclusions: Based on the results, HEMA concentrations of 15%, 20%, and 30% in our experimental bonding agent had no effect on the microleakage of class V restorations. They were not significantly different in terms of degree of conversion either.

Keywords: Photoinitiator, Microleakage, Degree of Conversion, Dentin Bonding Agent

1. Background

Dental adhesives are used mainly for bonding restorative materials to the tooth structure (1, 2). The mechanism of action of recent dentin bonding agents is based on the resin's penetration into the dentin structure (3-5), which forms the micromechanical retention between the adhesive resin and the etched dentin surface (6-8).

Currently, most of the recent dentin bonding agents available in the market contain fillers because the incorporation of fillers into the adhesive system can improve the mechanical properties of the adhesive layer, which enhances bond strength (9-15).

Although the addition of nanoclay particles into diluted systems, such as dental adhesives, results in the fast deposition of particles due to their high density, the surface modification of nanoclay particles with a polymer will eliminate this problem by decreasing the density of particles and by mediating reactions between active groups that are freshly adhered to the particle surface and the solvent. Monomer grafting is an effective method to create active groups on the surface of nanoparticles (16-18). Evidence shows that grafting nanoclay particles with polyacrylic acid, polymethacrylic acid, and polymethyl methacrylic acid monomers enhances the mechanical properties of adhesives (14, 16, 19).

Hydroxyethylmethacrylate (HEMA) is a small monomer that is present in the composition of most dentin bonding agents. Unpolymerized HEMA is in liquid form and is well soluble in water, ethanol, and acetone. Moreover, HEMA can evaporate from an adhesive liquid only in small amounts. Hydrophilicity is an important characteristic of HEMA. Although HEMA cannot be used as a demineralizing agent, it promotes adhesion due to its hydrophilicity. HEMA significantly increases bond strength by improving the wettability of dentin. However, HEMA will easily absorb water in both unpolymerized and light-polymerized forms. Jacobson and Soderholm speculated that adhesives containing HEMA are more susceptible to water contamination. HEMA in an unpolymerized adhesive may absorb water and dilute monomers to a level that inhibits polymerization. The amount of HEMA present in polymerized polymer chains allows it to maintain its hydrophilicity, which results in water sorption and consequent swelling and discoloration. Aside from water sorption, which negatively affects the mechanical strength, high concentrations of HEMA yield

flexible, low-quality polymers; poly-HEMA is basically a porous, flexible polymer (gel). Thus, high concentrations of HEMA in adhesives may adversely affect the mechanical properties of a polymer. HEMA decreases the water vapor pressure and probably the alcohol vapor pressure. Thus, high concentrations of HEMA may prevent adequate evaporation of solvent from an adhesive.

Similar to all methacrylates, HEMA is susceptible to hydrolysis, especially at a basic pH. It is also susceptible to hydrolytic degradation at an acidic pH. Currently, HEMA is widely incorporated into the composition of adhesives not only to ensure good wettability but also as a solubilizing agent because of its solvent nature. This characteristic improves the stability of solutions containing both hydrophobic and hydrophilic components and maintains their balance in a solution state (20).

Bonding to enamel and dentin by experimental dentin bonding agents containing nanoclay particles has shown high values of microleakage. Mousavinasab et al. reported that a possible reason for this high microleakage, despite a stronger bond, was the presence of a high concentration of HEMA (26%) in the composition of an experimental bonding agent compared to the control adhesive, adper single bond, which contained 10% - 20% HEMA (21). Since no previous study has evaluated the effect of HEMA concentration on microleakage and degree of conversion.

2. Objectives

This in vitro study sought to assess the effect of concentration of HEMA in an experimental dentin bonding agent on the degree of conversion of adhesive and the microleakage of class V composite restorations bonded with an experimental dentin bonding agent.

3. Methods

3.1. Fabrication of Experimental Dentin Bonding Agent

3.1.1. Bonding Polyacrylic Acid to Sodium Nanoclay

Hydrophilic sodium nanoclay (Na-nanoclay) (Cloisite Na^+, montmorillonite, Cloisite, USA) was used in this study. First, 5 - 10 g of Na-nanoclay was added to a glass reactor containing 1 L of water and was stirred at 50°C for 12 hours to separate the nanoclay sheets in an aqueous environment. The temperature of the reactor was then increased to 70°C, 2.5 g of 2-Acrylamido-2-methyl-1-propanesulfonic acid was added as an inactive surfactant, and the mixture was ultrasonicated (Sonoplus UW2200, Bandelin, Germany) for three minutes. Ammonium persulfate (as an initiator), a polyacrylic acid monomer, and a tertiary dodecyl mercaptan transfer agent were added to control the molecular weight of the polymer chains. The reaction was continued while being mixed for one hour. After completion of the reaction, the mixture was coagulated using 1% - 2% aluminum sulfate, and the compound was subjected to Soxhlet extraction using water as a solvent. By doing so, hemopolymers not bonded to the surface of particles were eliminated. The Soxhlet extraction product was placed in a vacuum oven at room temperature for 24 hours. The obtained dry material was ground in a ball mill (Germany PM100, RetchT). The obtained powder was filtered using a 400-mesh filter and was then ready to be added to the adhesive (14, 16, 19).

3.1.2. Addition of Grafted Nanoclay to the Adhesive Liquid

Grafted nanoclay was added to the adhesive liquid, and the mixture was ultrasonicated with 50% power for three minutes using six pulses of one second. The experimental bonding agent with the formulation presented in Table 1 was fabricated in the Iranian polymer research center.

Table 1. Composition of the Experimental Adhesive Used

Materials	Weight, %
Bis[4-(2-hydroxy-3-methacryloyloxyproxy)phenyl] propane (Bis-GMA)	14
2-Ethyl-2-(hydroxymethyl)-1,3-propandiol trimethacrylate	8
Urethane dimethacrylate	12
Ethanol	40

Nanoclay particles grafted with polyacrylic acid in 0.2 wt% and HEMA with three different concentrations were added to the experimental bonding agent to obtain three compositions:

Composition 1: The experimental dentin bonding agent containing 15% HEMA.

Composition 2: The experimental dentin bonding agent containing 20% HEMA.

Composition 3: The experimental dentin bonding agent containing 30% HEMA.

3.2. Tooth Preparation

Sound-extracted premolar teeth (which were extracted for orthodontic or periodontal reasons) were collected and stored in 10% formalin. Their buccal and lingual surfaces were evaluated under a stereomicroscope to ensure absence of caries, fracture, wear, crack, or developmental anomalies. The teeth were randomly allocated to the study groups. Standard class V cavities measuring $2 \times 3 \times 3$ mm were prepared in the buccal and lingual surfaces of the teeth using a cylindrical diamond bur with a 1-mm diameter (Diatech, Scissdertal, Switzerland) under water coolant in such a way that the incisal margin of the cavity was 2

mm above the cementoenamel junction while the gingival margin was 1 mm below it. At the occlusal margin, enamel was beveled (45°C, 1 mm) in such a way that the occlusal margin of the cavity was 2 mm above the cementoenamel junction and the gingival margin was 1 mm below it. Each class V cavity was considered as one specimen, and a new bur was used after every five preparations. The cavity was etched with 37% phosphoric acid (3M, ESPE, USA) for 15 seconds followed by 10 seconds of rinsing. Drying was done in such a way that the dentin remained slightly moist.

The prepared specimens were randomly divided into three groups (each group included 20 specimens):

Group 1: The experimental dentin bonding agent with composition 1 was used.

Group 2: The experimental dentin bonding agent with composition 2 was used.

Group 3: The experimental dentin bonding agent with composition 3 was used.

For the bonding procedure, the respective adhesive in each group was applied by a microbrush in two thin layers, and after 30 seconds, the adhesive solvent was evaporated by a mild air spray for 15 seconds. Light curing was performed for 15 seconds by a light-curing unit (Demi LED Light Curing System Kerr Corp, Orange, CA, USA) with a light intensity of 450 mW/cm^2 for 20 seconds. The cavities were filled with a microhybrid light-curing composite (Filtek Z250, 3M ESPE, St. Paul, St. Paul, USA) in 1-mm increments. Each increment was light cured for 20 seconds, and finishing and polishing were carried out using Soflex disks (3M ESPE, St. Paul, St. Paul, USA). The specimens were then immersed in distilled water for one week in order to complete the polymerization.

3.3. Assessment of Microleakage

The specimens were subjected to 3000 thermal cycles in a digital thermocycler (Willytec, Version 3.0, Willytec GmbH, Greefelfing, Germany) at 55°C ± 5°C. After thermocycling, tooth apices were sealed with sticky wax and tooth surfaces were covered with two layers of nail varnish except for a 1-mm margin around the restoration. The specimens were immersed in a 10% methylene blue solution for 72 hours and were mounted in self-curing acrylic resin (Acropars, Kaveh, Tehran, Iran) for future cutting. The specimens were then sectioned buccolingually at the middle of the restoration by a double-sided diamond disc (NonStop, Albany, NY, USA).

Microleakage was assessed separately by two observers in a double-blind fashion under a stereomicroscope (Leica-ZOOM 2000, Wetzlar, Germany) at 20 × magnification and were scored from 0 - 4 at the occlusal and gingival margins:

0: No microleakage.

1: Dye penetration by one-third of the cavity depth.

2: Dye penetration by more than two-thirds of the cavity depth.

3: Dye penetration along the entire cavity depth.

4: Dye penetration along the entire cavity depth reaching the pulpal floor.

The sum of occlusal and gingival margin microleakage scores was defined as the microleakage score for each specimen.

3.4. Measurement of the Degree of Conversion of the Experimental Adhesive

One drop of each adhesive (in the three groups) was placed on a thin polyethylene sheet. The solvent was gently evaporated by mild airflow for 15 seconds. To obtain a very thin layer of adhesive, a second sheet was applied, the sample was subjected to Fourier transform infrared spectroscopy (FTIR) (Perkin Elmer, Spectrum GX, USA), and the absorbance was read in transmission mode before and after light curing. Degree of conversion was calculated based on the ratio of the optical density of the C=C aliphatic group (maximum at 1638 cm^{-1}) divided by the internal reference of the C=C aromatic group (maximum at 1608 cm^{-1}) before and after curing.

$$DC\,(\%) = \left(1 - \frac{\left(\frac{1638cm^{-1}}{1608cm^{-1}}\right) Peak\ \ area\ \ after\ \ curing}{\left(\frac{1638cm^{-1}}{1608cm^{-1}}\right) Peak\ \ area\ \ after\ \ curing}\right) \times 100 \qquad (1)$$

The data was analyzed using SPSS, and the mean and standard deviation (SD) values were reported for the degree of microleakage and the degree of conversion. The Kruskal-Wallis test was used to compare the microleakage of the groups. Fisher's exact test was applied to compare the groups in terms of occlusal microleakage. The Wilcoxon test was used to compare occlusal and gingival microleakage within each group. The Kruskal-Wallis test was used to compare the degree of conversion among the groups and was followed by a post hoc Mann-Whitney test. $P < 0.05$ was considered statistically significant.

4. Results

Table 2 shows the frequency distribution and the results of the Kruskal-Wallis test that was used to compare the total microleakage of the studied groups, which revealed no significant difference ($P > 0.05$).

Table 3 shows the frequency distribution of the degree of microleakage at the occlusal margin of the restorations in the studied groups.

The Kruskal-Wallis test did not find any significant difference between the groups regarding microleakage at the occlusal margin ($P > 0.05$).

Table 2. Frequency Distribution of the Degree of Total Microleakage in the Studied Groups[a,b]

Study Group	Degree of Microleakage						P Value
	0	1	2	3	4	Total	0.37
Group 1 (15% HEMA)	21 (52.5)	9 (22.5)	5 (12.5)	4 (10)	1 (2.5)	40 (100)	
Group 2 (20% HEMA)	27 (67.5)	6 (15)	6 (15)	1 (2.5)	0 (0)	40 (100)	
Group 3 (30% HEMA)	25 (62.5)	6 (15)	2 (5)	5 (12.5)	2 (5)	40 (100)	

[a] Values are expressed as No. (%).
[b] Kruskal-Wallis test.

Table 3. Frequency Distribution of the Degree of Microleakage at the Occlusal Margin of the Restorations in the Studied Groups[a,b]

Study groups	Degree of Microleakage						P Value
	0	1	2	3	4	Total	1
Group 1 (15% HEMA)	19 (95)	1 (5)	0 (0)	0 (0)	0 (0)	20 (100)	
Group 2 (20% HEMA)	19 (95)	1 (5)	0 (0)	0 (0)	0 (0)	20 (100)	
Group 3 (30% HEMA)	20 (100)	0 (0)	0 (0)	0 (0)	0 (0)	20 (100)	

[a] Values are expressed as No. (%).
[b] Kruskal-Wallis test.

Table 4 presents the frequency distribution of the degree of microleakage at the gingival margin of the restorations in the studied groups.

The Kruskal-Wallis test did not find any significant difference between the groups regarding microleakage at the gingival margin ($P > 0.05$).

Within each group, microleakage at the occlusal and gingival margins was significantly different ($P < 0.05$, Table 5).

Table 6 presents the mean and SD of the degree of conversion in the three groups.

According to the Kruskal-Wallis test, the degree of conversion of the three groups was not significantly different ($P > 0.05$).

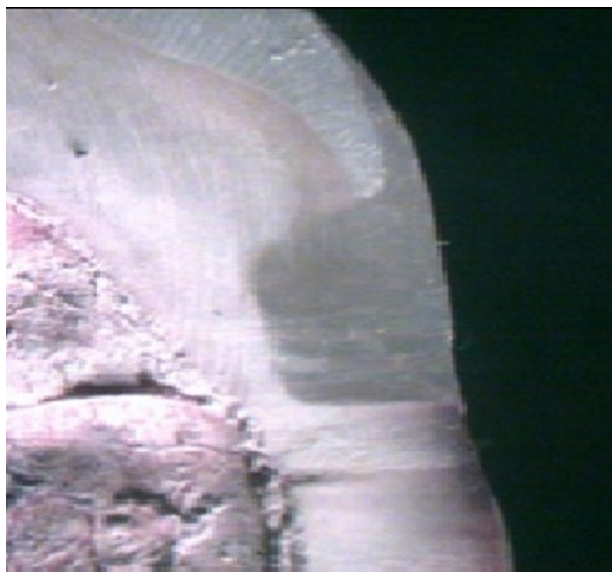

Figure 1. Grade 0 Microleakage at the Gingival Margin

5. Discussion

Clinically, achieving the highest degree of conversion possible in the materials used in esthetic dentistry has always been desirable since a higher degree of conversion results in higher strength. Although a higher degree of conversion increases shrinkage and tensile stresses, inadequate polymerization causes more significant problems, such as lower biocompatibility, lower mechanical and physical properties, and early potential failure of restorations (20).

Currently, failure of the bond of restorative materials to enamel and dentin remains a major problem. The presence of microscopic gaps at the interface of the restorative material and the cavity walls enables the penetration of ions, molecules, bacteria, and fluid into the cavity and causes postoperative tooth hypersensitivity, marginal staining of restoration, secondary caries, and pulpal inflammation (22).

The final goal of the application of dentin bonding agents is to achieve a strong and durable bond between the tooth and restorative material. In the current study, a dentin adhesive containing filler was used because studies have shown that the addition of fillers into adhesives enhances their mechanical properties and bond strength. Accordingly, Kim et al. indicated that the addition of

Table 4. Frequency Distribution of the Degree of Microleakage at the Gingival Margin of the Restorations in the Studied Groups[a,b]

Study groups	Degree of Microleakage						P Value
	0	1	2	3	4	Total	0.181
Group 1 (15% HEMA)	2 (10)	8 (40)	5 (25)	4 (20)	1 (5)	20 (100)	
Group 2 (20% HEMA)	8 (4)	5 (25)	6 (30)	1 (5)	0 (0)	20 (100)	
Group 3 (30% HEMA)	5 (25)	6 (30)	2 (10)	5 (25)	2 (10)	20 (100)	

[a] Values are expressed as No. (%).
[b] Kruskal-Wallis test.

Table 5. Comparison of Microleakage at the Occlusal and Gingival Margins Within Each Group[a]

Study Groups	Z-Wilcoxon	P Value
Group 1 (15% HEMA)	-3.67	< 0.001
Group 2 (20% HEMA)	-3.002	0.003
Group 3 (30% HEMA)	-3.45	0.001

[a] Wilcoxon test.

Table 6. Comparison of Degree of Conversion Among the Studied Groups[a]

Study Groups	Mean (%)	Standard Deviation	Degree of Freedom	P Value
Group 1 (15% HEMA)	53.36	3.34	2	0.733
Group 2 (20% HEMA)	58.9	10.57		
Group 3 (30% HEMA)	60.7	23.32		

[a] Kruskal-Wallis test.

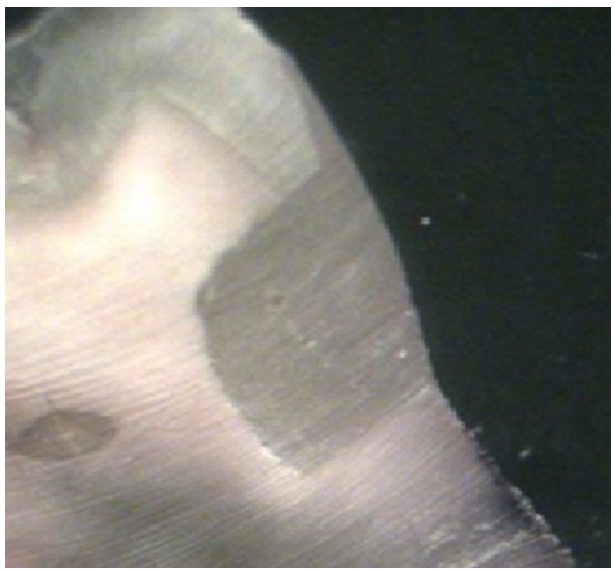

Figure 2. Grade 1 Microleakage at the Gingival Margin

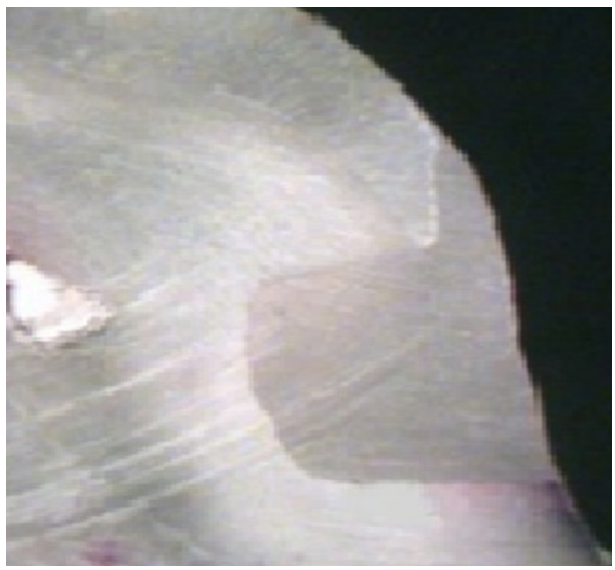

Figure 3. Grade 2 Microleakage at the Gingival Margin

nanofillers to dental adhesives in a less than 1 wt% concentration increases microtensile bond strength. In the current study, montmorillonite clay nanoparticles were used (23). The incorporation of these nanoclay particles in polymer systems causes significant improvement of mechanical properties, and these particles are inherently hy-

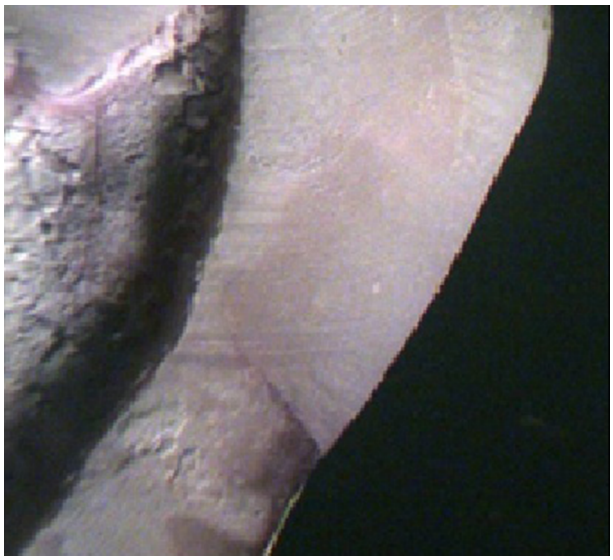

Figure 4. Grade 3 Microleakage at the Gingival Margin

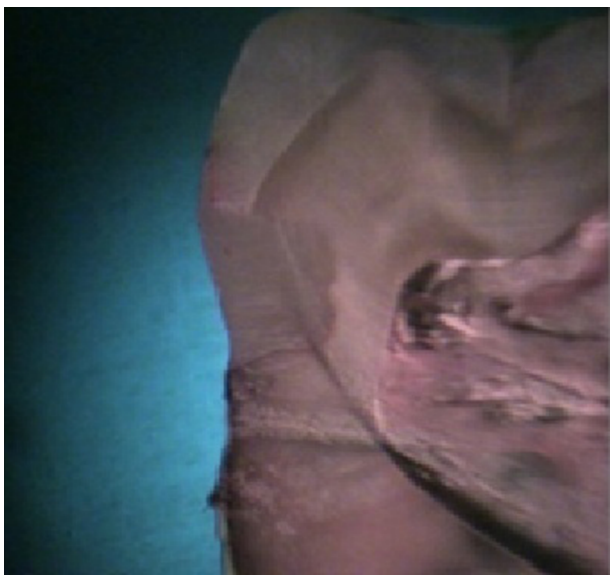

Figure 5. Grade 4 Microleakage at the Gingival and Occlusal Margin

drophilic (16, 23).

The amount of photoinitiator added to dentin bonding agents depends on the type of initiator and type of bonding system used, but they are often incorporated in very small amounts (0.1% - 1%). In the current study, a 1 - 1.5 wt% photoinitiator was added to the composition of the experimental adhesive (20).

Several methods have been proposed to determine the efficacy of the light polymerization of dental resins, such as the use of nuclear magnetic resonance, FTIR, and high-performance liquid chromatography. In the current study, FTIR was used because this method is highly efficient for the molecular detection and assessment of chemical reactions and because this method provides a better interpretation of changes that occur in a polymer matrix (24, 25). This method was also used by Solhi et al. (14), Park et al. (26), and Shin et al. (27) for the assessment of the efficacy of the polymerization of bis-GMA composites.

Currently, the dye penetration technique is the most commonly used method for the assessment of microleakage because it has appropriate technic sensitivity and is simpler than other methods. Moreover, this technique does not require the use of hazardous equipment and materials (28). Thus, in the current study, the dye penetration method with 10% methylene blue was used for this purpose. The only drawback of this method is its scoring system since its accuracy decreases with reevaluation after a time interval or assessment by more than one observer (22).

Based on the current results, the degree of conversion of adhesive was enhanced with an increase in concentration of HEMA, though the difference in this enhancement between the groups was not statistically significant. Van Landuyt showed that in a self-etch experimental adhesive, 10% concentration of HEMA increased bond strength; however, with higher concentrations of HEMA, a reduction in bond strength was noted due to the low degree of conversion and formation of drops in the adhesive structure (29).

HEMA is a monofunctional monomer, and its polymerization rate is lower than multifunctional monomers. To achieve adequate and homogenous HEMA polymerization, 600 seconds of light irradiation is required (30). Thus, higher concentrations of HEMA in the composition of an adhesive may decrease the polymerization of polymer and its degree of conversion. However, the results of these studies did not confirm our findings.

Our study also showed no significant difference in the microleakage of adhesives at the enamel or gingival margins with different concentrations of HEMA. Based on in vivo and in vitro studies, factors preventing an effective bond between the adhesive and dentin include the water sorption and hydrolysis of adhesive resin, inadequate conversion of monomer/polymer penetrated into dentin, low penetration of resin into dentin, and insufficient evaporation of solvent (31). Fortin et al. (32) and Mousavinasab et al. (21) reported results similar to those of the current study. A comparison of microleakage at the occlusal and gingival margins in each group showed a significant difference, which was in agreement with the results of Mine et al. (33), who reported higher bond strength in the enamel

margin irrespective of the concentration of HEMA. HEMA is added to the composition of an adhesive with the aim of enhancing the bond and adhesive properties in dentin. In the enamel, with the absence of the tubular fluid and the collapse of the collagen fibers, an adequate bond is provided by adhesive penetration into microporosities in the structure of etched enamel.

Manhart et al. (34) demonstrated that marginal microleakage in class V restorations was significantly different in the occlusal and gingival margins in use in different dentin bonding systems, which was in agreement with our findings. Additionally, in vitro studies revealed that microleakage at the gingival margin of class II restorations was higher than that at the proximal walls (31).

In order to provide ideal conditions and to better simulate the clinical setting, similar studies are required that employ a higher frequency of thermal cycles, which corresponds with longer clinical service, or on samples under masticatory function in the oral environment for some time. The measurement of microleakage along with the use of a fatigue test for the aging of samples are also recommended.

In the current study, FTIR was used to assess the degree of conversion. The assessment of degree of conversion with FT-Raman spectroscopy or micro-Raman spectroscopy and the comparison of their results with those of FTIR may better elucidate this topic. The duration of solvent evaporation was not evaluated in the current study. Future studies are required to study the role of this factor. Additionally, the assessment of the microleakage of this experimental adhesive with other concentrations of HEMA is recommended to find the most efficient concentration of HEMA in the adhesive composition.

5.1. Conclusion

Within the limitations of this study, the results showed that 15%, 20%, and 30% concentrations of HEMA in the experimental bonding agent had no significant effect on the microleakage of class V composite restorations. These agents were also not different in terms of degree of conversion.

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Footnotes

Authors' Contribution: Drafting of the manuscript, critical revision of the manuscript for important intellectual content Farnaz Monsef Esfahani; study concept and design, analysis and interpretation of data, study supervision, statistical analysis, Zahra Khamverdi; administrative, technical, and material support, Mohammad Ataei; acquisition of data, Soudabeh Ebrahimi.

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