

Effect of 2,3-Butanedione in an Experimental Dentin Bonding System on Degree of Conversion and Microleakage of Class V Composite Restorations

Zahra Khamverdi¹, Soodabe Ebrahimi^{2*}, Mohammad Atai³ and AliReza Soltanian⁴

¹Professor, Dental research center, Hamadan University of Medical Sciences, Iran

²Post graduate student, Department of Prosthodontics, Iran

³Associate Professor, Iran Polymer and Petrochemical Institute, Tehran, Iran

⁴Professor, Department of Biostatistics, Health school, Iran

*Corresponding author: Ebrahimi Soodabe, Postgraduate student of Prosthodontics, Department of Prosthodontics, Dental school, Hamadan University of Medical Sciences, Hamadan, Iran



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Abbreviations: CQ/amine: Camphorquinone /Amine, DC: Degree of Conversion, FTIR: Fourier transform infrared spectroscopy, BD: butanedione, PAA-g- nano clay: Poly Acrylic Acid Grafted with Nano clay

ABSTRACT

Objective(s): Using clay nanoparticles grafted with polyacrylic acid in the recent studies has shown significant improvement in bond strength. Incorporation of these materials into the experimental adhesive suggests the theory that adhesives containing active acidic groups deactivate the amine group and decrease the degree of conversion of adhesives with Camphorquinone (CQ) /amine photo initiator and resulting in higher microleakage.

Materials and Methods: 60 class V cavities were prepared on the buccal/lingual surfaces of extracted, sound premolar teeth and restored using an experimental dentin bonding agent and light-cure composite in three groups (n=20): Group1: 1wt% CQ/amine, Group2: 1wt%butanedione and Group3: 0.5wt% CQ+1wt% butanedione. Microleakage was assessed at the occlusal and gingival margins of the restorations. degree of conversion of the adhesive was calculated using Fourier transform infrared spectroscopy. Data were analyzed using SPSS23 and Kruskal Wallis, Wilcoxon, Mann Whitney U and Fisher's exact tests ($\alpha=0.05$).

Results: Score of microleakage was not significantly different at the occlusal and gingival margins among the groups but it was significantly different at the occlusal margin compared to the gingival margin in all groups ($P<0.001$). Degree of conversion of groups 1 and 3 was significantly different from that of group 2; but groups 1 and 3 were not significantly different ($P=0.027$).

Conclusion: Using 1wt% butanedione instead of 1wt% CQ/amine in the understudy dentin adhesive system or the combination of both had no significant effect on microleakage of composite restorations. degree of conversion of dentin adhesive containing butanedione was significantly lower than other groups.

Introduction

Recent advances in dental adhesives and composite resins are responsible for the popularity of these materials for direct restoration of carious lesions [1]. At present, bond to dentin is weaker than the bond to enamel due to the heterogeneous and moist nature of dentin. Thus, a long-lasting, durable bond to dentin

is less commonly achieved compared to enamel [2,3]. Of all the components involved in bonding at the resin-dentin interface, the adhesive layer has the lowest modulus of elasticity [4,5]. Thus, most adhesive systems available in the market contain fillers that increase the mechanical properties and the bond strength of the restorations [6-9].

Nanometer-sized fillers like clay are available; By performing adequate surface treatments, these fillers can be lodged in between polymer chains in dentin adhesive layer and increase the mechanical properties of the adhesive and subsequently enhance the bond strength [10,11].

Addition of clay nanoparticles to dentin bonding agents results in fast deposition of particles due to their higher density. However, this problem can be solved by surface treatment of nano clay particles with a polymer, which decreases density and results in a reaction between active groups bonded to the surface of particles and the solvent. One effective method to form active groups on the surface of nanoparticle is their grafting with monomers [4,5,12]. Studies on this topic have demonstrated that grafting nano-clay particles with poly acrylic, poly methacrylic and poly methyl methacrylic acids enhances the mechanical properties of adhesives [12-14].

Using clay nanoparticles grafted with polyacrylic acid in the recent studies has shown significant improvement in bond strength [14]. Incorporation of these materials into the experimental dentin bonding agent suggests the theory that adhesives containing active acidic groups deactivate the amine group and decrease the degree of conversion of adhesives with CQ/amine photo initiator resulting in a weak bond between dentin and composite and allowing higher diffusion of water at the composite/dentin interface and subsequently higher microleakage. To overcome this problem, a single step photo initiator like 2,3 butanedione can be used.

In the recent studies, several types of new photo initiators such as 2,3 butanedione, 1-phenyl-1,2-propanedione and hexafluoroantimonate p-octyloxy-phenyl iodonium have been evaluated [15,16]. CQ is the most commonly used photo initiator in light-cure composite resins [15]. CQ is naturally yellow causing problems in color matching. This limits the application of its higher concentrations and consequently, the DC and curing depth [15]. The 2,3 butanedione (Diketone BD) is a viscous liquid. Due to its physical state, it is well compatible with resin components and in contrast to CQ, which is solid at room temperature, it can be used as a diluting agent in composite resins. The 2,3 butanedione is a photo initiator without the amine activator. By light irradiation and cleavage of C-C bond, it produces two carbonyl free radicals and initiates polymerization [15,17]. In the UV -VIS spectrum, butanedione has a peak absorbance at 419 nm, which is close to the output power of dental light curing units. On the other hand, shorter absorbance wavelength of butanedione lowers its yellowness compared to that of CQ [15]. The aim of the present study was to evaluate the effect of type of photo-initiator (2,3 butanedione, CQ/amine or both) in an experimental dentin bonding agent on the DC of adhesive and microleakage of class V composite restorations bonded with this experimental dentin bonding agent.

Materials and Methods

The graft polymerization of acrylic acid onto the surface of nanoclay was carried out according to the previous works [14].

The grafted nanoclay powder was added to the adhesive solution and the obtained mixture was sonicated for 3 minutes under 50% power and 6 pulses (Each pulse for 1 second). Dentin adhesive system was manufactured in the Iranian Polymer Research Center according to the formulation presented in Table 1. Polyacrylic acid-grafted nano clay filler particles were added by 0.2wt%. Photo initiator was added as follows:

Table 1: The composition of the experimental bonding agent.

Materials	%Weight
Bis[4-(2-hydroxy-3-methacryloyloxyproxy) phenyl] propane (Bis-GMA)	14
2-Hydroxy ethyl methacrylate (HEMA)	26
2-Ethyl-2-(hydroxymethyl)-1,3-propandiol trimethacrylate (TMPTMA)	8
Urethane dimethacrylate (UDMA)	12
Ethanol	40

Formulation 1: 1wt% CQ/amine

Formulation 2: 1wt% butanedione

Formulation 3: 1wt% butanedione+0.5wt%CQ

Preparation of Teeth

30 Sound human premolar teeth were collected and stored in 10% formalin. Their buccal and lingual surfaces were examined under a stereomicroscope for presence of any caries, cracks, abrasion, fracture, or developmental anomalies. Using a cylindrical diamond bur measuring 1mm in diameter (Diatech, Scissortail, Switzerland) along with water and air spray, standard class V cavities measuring 2×3×3 mm were prepared on the buccal and lingual surfaces of teeth; Enamel at the occlusal margin was beveled (45°, 1mm). Each class V cavity was considered as one specimen and the bur was discarded after preparation of 5 cavities. The cavities were etched using 35% phosphoric acid gel (3M ESPE, St Paul, MN,USA) for 15 seconds and were then rinsed with water for 10 second and dried leaving the dentin surface relatively moist. Prepared specimens were randomly divided into three groups (n= 20):

1: The experimental dentin bonding agent with 1wt% CQ/amine,

2: The experimental dentin bonding agent with 1wt% butanedione and

3: The experimental dentin bonding agent with 1wt% butanedione+0.5wt%CQ

The adhesive in each group was applied to the cavity surfaces using a micro brush in two thin coats and after 30 seconds, the adhesive solvent was evaporated with gentle air spray for 15 seconds. Light curing was performed using Spectrum 800 halogen light-curing unit (Dentsply/Caulk, USA) for 20 seconds. The cavities were filled using a microhybrid light cure composite (Filtek Z250, 3M ESPE, St. Paul, USA) in 1-mm increments. Each increment

was light cured for 20 seconds and finishing and polishing were performed using Soflex burs (Passanti 332, Kerr, Italy-Switzerland).

Measurement of Microleakage

Specimens were subjected to 3000 thermal cycles using digital thermocycler (Willytec version 3.0, Willytec GmbH, Gräfelfing, Germany) at 5-55°C. After thermocycling, tooth apices were sealed with sticky wax and the surfaces of the teeth were coated with two layers of nail varnish except for one-millimeter margin around the cavities. Specimens were immersed in 10% methylene blue for 72 hours and were mounted in self-curing acrylic resin (Acropars, Kaveh, Tehran, Iran) for future cutting. Using a diamond disc, specimens were buccolingually sectioned at the middle of the restoration using a cutting machine (Nonstop, Albany, NY, USA).

Microleakage of each specimen was measured using a stereomicroscope (Leica Zoom 2000, Wetzlar, Germany) at 20X magnification separately by two observers in a double-blind fashion. The score of microleakage at the occlusal and gingival margins was scored using the following scoring system [18]:

- 0: No microleakage
- 1: Dye penetration to 1/3 of cavity depth
- 2: Dye penetration to 2/3 of cavity depth
- 3: Dye penetration to the entire depth of cavity
- 4: Dye penetration exceeding the depth of cavity towards the pulp

The mean score of microleakage at both the occlusal and gingival margins was considered as the microleakage score for the respective specimen.

Measurement of the DC of the Three Formulations of the Experimental Adhesive

One drop of each formulation of the experimental adhesive was placed on a thin polyethylene sheet. The solvent was gently vaporized using mild air spray for 15 seconds. To obtain a very thin coat, a second sheet was placed over it and the complex was transferred to a FTIR spectrophotometer (Spectrum GX, Perkin Elmer). The absorbance of the sample before and after light curing was measured using the transmission mode of FTIR. DC was calculated based on the ratio of the absorption intensity of C=C aliphatic group (absorption peak height at 1638cm⁻¹) divided by the internal reference of C=C aromatic group (absorption peak height at 1608cm⁻¹) before and after curing of the specimen [18].

$$DC (\%) = (1 - \frac{I_{1638}}{I_{1608}}) \times 100$$

Data of the score of microleakage and DC were entered in

SPSS software and the mean and standard deviation values were calculated. Kruskal Wallis test was used to compare the degree of microleakage between groups. Using Fisher's exact test, occlusal microleakage was compared between groups. For intragroup comparison of microleakage between the occlusal and gingival margins, Wilcoxon test was applied. To compare DC between groups, Kruskal Wallis and Mann Whitney U tests were used. Significance level was set at 0.05.

Results

Data for scoring of microleakage in the study groups is reported in Table 2. The results of Kruskal Wallis test for comparison of microleakage between groups revealed no significant difference (P=0.64). Table 3 shows the score of microleakage in the study groups at the occlusal and gingival margin of restorations. Fisher's exact test compared the microleakage at the occlusal margin and found no significant differences between groups (P=0.52), the Kruskal Wallis test compared the microleakage at the gingival margin and found no significant difference in this respect among groups (P=0.76). Comparison of microleakage at the gingival and occlusal margins (Figure 1) in each study group revealed a significant difference (P<0.05). The mean Degree of conversion for the first group (1wt% CQ/amine) was 53.04±2.98 %, and 14.66±0.57 % in the second group (1wt% butanedione), and 44.48±5.94 % in the third study group (1wt% butanedione+0.5wt%CQ). Based on the results of the Kruskal Wallis test, DC was significantly different between the study groups (Table 4). Pairwise comparison of the study groups for degree of conversion, by Mann Whitney U test indicated a significant difference between groups 1 and 2 (p=0.046), and between groups 2 and 3 (p=0.046). However, the difference between groups 1 and 3 was not significant (p=0.055).

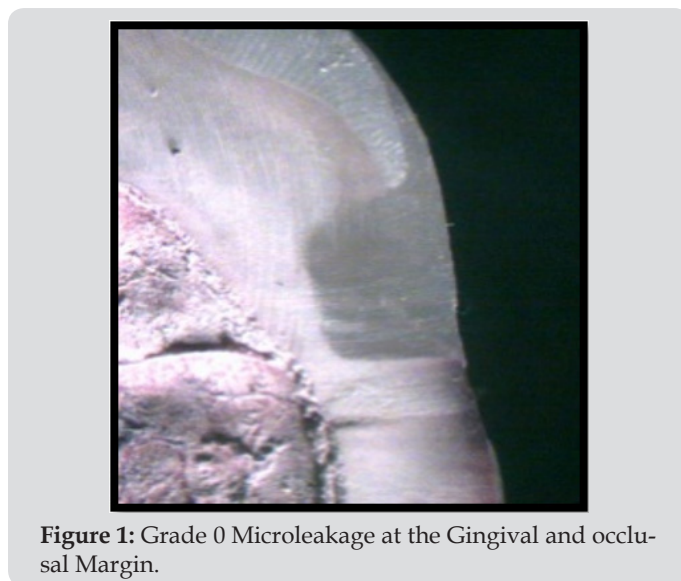


Figure 1: Grade 0 Microleakage at the Gingival and occlusal Margin.

Table 2: Frequency Distribution of the Degree of Total Microleakage in the Studied Groups^{a,b}.

Study Groups	Score of microleakage						P value
	0	1	2	3	4	Total	
Group1 (1wt% CQ/amine)	19(47.5)	8(20)	6(15)	5(12.5)	2(5)	40(100)	0.64
Group2 (1wt% butanedione)	22(55)	9(22.5)	4(10)	5(12.5)	0	40(100)	
Group3 (1wt%butanedione+ 0.5wt% CQ)	20(50)	10(25)	7(17.5)	3(7.5)	0	40(100)	

Note: aValues are expressed as No. (%).

bKruskal-Wallis test.

Table 3: Score of microleakage at the occlusal/gingival margin of restorations in the study.

Study Groups	Score of Microleakage									
	0		1		2		3		4	
	O*	G**	O	G	O	G	O	G	O	G
Group1(1wt% CQ/amine)	16	3	2	6	1	5	0	5	1	1
Group2(1wt% butanedione)	18	4	2	7	0	4	0	5	0	0
Group3 (1wt%butanedione+0.5wt% CQ)	19	1	0	10	1	6	0	3	0	0

Table 4: Comparison of Degree of conversion among the study groups*.

Study Groups	Mean (%)	Standard Deviation	Degree of freedom	P value
Group1	53.04	2.98	2	0.027
Group2	14.666	0.577		
Group3	44.486	5.944		

Note: * Kruskal - Wallis

Discussion

Microleakage is a factor responsible for pulpal reactions to restorative materials. Formation of microscopic gaps at the tooth-restoration interface enables the passage of ions, molecules, bacteria and their toxic products as well as liquids, causing post-operative tooth hypersensitivity, marginal discoloration, secondary caries and pulp inflammation [19-21]. Degree of conversion (DC) is an important criterion for clinical success of a composite resin restoration. Clinically, polymerization of materials used in esthetic dentistry must be increased as much as possible to achieve the maximum restoration strength. Although increased DC increases the polymerization shrinkage of adhesive or restoration and subsequently imparts tensile stresses to the bonded interface, it can be compensated by application of suitable clinical technique; while inadequate polymerization causes problems such as less biocompatibility, lower physical and mechanical properties and early failure of the restoration [22].

In the current study, a filler-containing dentin adhesive was used. Studies have reported that filler-containing adhesives have improved mechanical properties and enhance the bond strength [6-9,13,14]. Kim et al. [23] reported that addition of less than 1wt% nanofillers to dental adhesives increased the microtensile bond strength of the composite restoration; but in higher amounts of nanofillers, the microtensile bond strength decreased due to the aggregation of particles and formation of clusters. Solhi et al. [14] demonstrated that dental adhesives containing 0.2 wt% PAA-g-

nano clay particles, showed a significantly increased bond strength. The photo initiator is often added in the range of 0.1-1% [22]. In the present study, we applied 1-1.5wt% of photo initiator within the formulation of the experimental adhesive.

There are some relevant methods to determine the degree of monomer to polymer conversion of composite resin specimens like FTIR spectroscopy, Laser Raman spectroscopy, Electron Spin Resonance (ESR), Infrared Spectroscopy (IR), Dynamic Mechanical Thermal Analysis (DMTA), Attenuated Total Reflection (ATR) etc [24]. In the current study, FTIR was used because it is a beneficial method for molecular detection and tracing of chemical reactions. It provides a better interpretation of the changes that occur in the polymer matrix. FTIR can easily detect and quantify chemical bonds [25]. This method has been used by Solhi et al. [14], Park et al. [26], Yazdi et al. [27] and Shin et al. [16] to assess the polymerization efficacy of Bis-GMA-based composite resins. Dye penetration is the most commonly used technique for assessment of microleakage. This method has acceptable technical sensitivity and is easier than other methods available for this purpose [28]. Thus, researchers use fuchsin or methylene blue with particles equal in size or smaller than the size of bacteria (about 2 microns) [19]; In the current study, 10% methylene blue was used. The only drawback of this method is its grading system because its sensitivity decreases by re-evaluation after a time interval [19]. *In-vitro* studies on microleakage are often performed on class V restorations. Specimens are usually subjected to thermocycling

or mechanical loading to simulate the oral environment [28]; In the current study, specimens were subjected to 3000 thermal cycles prior to microleakage assessment. The results showed that in all groups some degrees of microleakage existed and none of the formulations evaluated in this study could provide complete marginal seal. No significant difference was seen among the study groups in terms of microleakage.

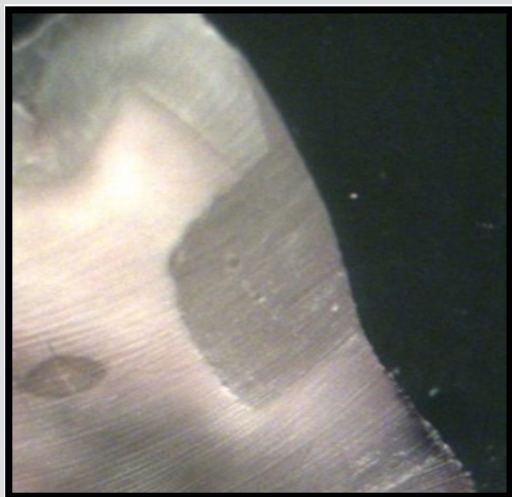


Figure 2: Grade 1 Microleakage at the Gingival Margin.

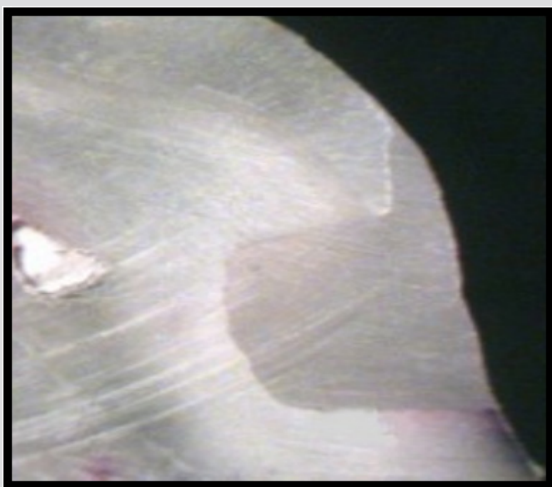


Figure 3: Grade 2 Microleakage at the Gingival Margin.

Fortin et al. [29] compared microleakage in several dental adhesive systems and reported that some degrees of microleakage were detected in all groups; which is in accord with the current study results. According to a study by Mousavinasab et al. [18] score of microleakage was not significantly different in several experimental filler-containing adhesives and Single Bond. This finding is in line with our results. Our study also showed that in all groups, microleakage at the gingival margin (Figures 2-5) was significantly higher than that in the occlusal margin; This indicates that marginal seal in the dentin margin was lower than that in the enamel margins. Lower microleakage in the enamel compared to dentin margin is due to the stronger bond to enamel because the enamel has a higher mineral content and is more uniformly

mineralized compared to dentin. Also, dentinal fluid does not exist in enamel and thus, monomers can more easily penetrate its microporosities created following etching, resulting in a more reliable micromechanical bond. Another reason for the lower microleakage in the enamel margin is beveling the enamel margin increases the bondable enamel surface [20].

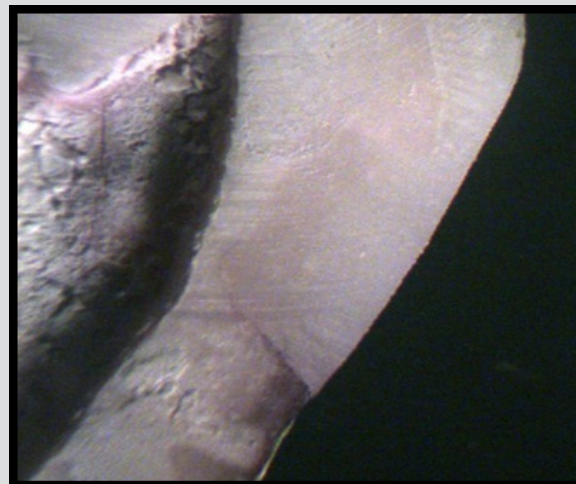


Figure 4: Grade 3 Microleakage at the Gingival Margin.



Figure 5: Grade 4 Microleakage at the Gingival and occlusal Margin.

Also, by exposure of enamel crystals, a stronger bond is achieved. Manhart et al. [30] demonstrated that marginal microleakage in class V restorations bonded with several dentin bonding systems was significantly different at the occlusal and gingival margins; which also confirms our results. In the current study, DC was significantly different among the three groups and pair wise comparisons revealed significant difference of groups 1 and 3 with group 2 in this regard. However, groups 1 and 3 were not significantly different. Combining 1wt% 2, 3 butanedione and 0.5wt% CQ increased the DC compared to the use of 2,3 butanedione alone; It appears that using this combination as photo initiator (group 3) in the understudy experimental dentin bonding agent can be helpful because this formulation lacks linear

amine, which probably becomes inactivated in presence of acid. Thus, in combination of CQ and 2, 3 butanedione, the reaction for generation of free radicals with butanedione is usually done with CQ even when amine is not present in the formulation.

In the current study, incorporation of a combination of butanedione and CQ showed more favorable results. Thus, future studies are suggested to assess the properties of higher concentrations of butanedione in combination with CQ. Moreover, since the effect of duration of solvent vaporization was not investigated in the current study, further studies are required to assess the effect of this factor.

Conclusion

Under limitations of the present study, Substitution of 1wt% butanedione for 1wt% CQ/amine in the studied experimental adhesive system or the combination of butanedione and CQ had no effect on the microleakage of class V composite restorations. Also, DC of adhesive containing 1% butanedione was significantly lower than that of the adhesive containing 1% CQ or combination of both.

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Conflict of Interest

The study was funded by Vice chancellor for Research and Technology, Hamadan University of Medical Sciences.

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