The Effect of Three Mouthwashes on Micro Leakage of a Composite Resin-An in Vitro Study

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Abstract: Introduction: Using mouthwashes have been recommended for prevention and control of caries and periodontal diseases due to their affection on restorative dental materials. So, the purpose of this in vitro study was to investigate the effect of three mouthwashes of Chlorhexidine, Oral-B and Irsha on micro leakage of a composite resin. Methods: 56 class 5 cavities were prepared on buccal and lingual surfaces of 28 human molars. The cavity margins were placed 1mm occlusal and apical to CEJ, respectively. After restoring teeth with composite resin, they were randomly divided into four groups and immersed in solutions for 12 hours, then submitted to thermocycling. The specimens were coated with nail varnish, and then immersed in 2% basic fuchsin for 24 hours. Teeth were mounted in epoxy resin and sectioned buccolingually. Leakage scores were evaluated based on a standard ranking, under microscope (25x). Data were analyzed with Kruskal - wallis and Mann-Whitney tests at P<0.05. Results: degree of micro leakage between occlusal surfaces (P= 0.777) and between Gingival surfaces (P= 0.232) in four experimental solutions, was not significant. Also, Degree of micro leakage between occlusal and gingival of each group was not significant (P>0.05). Conclusion: The results of this study revealed that use of mouthwashes didn't increase micro leakage.

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1. Introduction:

During last decade, use of tooth-colored dental materials has been dramatically increased due to esthetic needs of patients[1]. The durability and longevity of the esthetic restorative materials are important factors in the oral environment for the proper selection of the material[2]. Composite resin that's one type of these materials possessing various benefits including esthetic, non-thermal conduction, no mercury-related side effects, adequate strength and bonding to tooth structure[3].

Based on latest studies, saliva, food components, beverages have significant affect dental composites[1].The use of mouthwashes has become popular recently as an effective method for prevention and control of caries and periodontal diseases. Furthermore, mouthwashes are widely used to reduce oral malodor as well as implant maintenance[4].

However, mouthwashes can impose detrimental effects on oral and dental tissues[5,6]. Alcohol in mouthwashes is used as solvent, taste enhancer as well as antiseptic agent[7].

Previous studies have indicated that alcohol in the mouthwashes can soften the composite resin restorations[8] thus decrease their hardness[9,10]. On the other hand, it has been reported that both alcohol-containing and alcohol-free mouthwashes adversely affect the hardness of the restorative materials[11].

However, one study found that essential oil contained mouthwashes has no deteriorative effects on restorative materials, even in the long-term use[12].

Fluoride mouthwashes can affect the solubility of some restorative materials[13,14]. In addition, sodium fluoride contained mouthwashes could discolor composite resins[10].

One of the important factors related to the success of restorative materials is their marginal integrity with dental tissue which leads to micro leakage. Micro leakage is the penetration of bacterium liquids, molecules and ions through the tooth-restorative materials interface. Micro leakage can create the sensitivity of the tooth, secondary caries formation, discoloration and pulp irritation[15].

Several studies have investigated the effects of various mouthwashes on the surface properties of composites[8-13,15-20]. However, to our knowledge few studies have been conducted about the effects of mouthwashes on micro leakage formation of restorative materials. Micro leakage of composite resin restorations are influenced by the type of adhesive system, mouthwash type and the marginal outline of the cavity[21,22].

Although, the effects of mouthwashes on restorative materials may be very different and influenced by many factors which are not easily investigated in vitro, but in vitro investigation of restorative materials is proposed for any new product[21].

Therefore, the purpose of the present study is to evaluate the effects of three mouthwashes (Chlorhexidine, Oral B and Irsha) on micro leakage of composite resin.

Materials and Methods:

Selection and Preparation of Teeth

28 of extracted human mandibular molars free of any caries, restorations, cracks or other defects were selected. The teeth were cleaned from any calculus or soft tissue debries by using an ultrasonic scaler and stored in physiologic saline with 0.05% sodium azide at 5°C for up to four month prior to use.

Preparation of Class V cavities on the buccal and lingual surfaces

Class V cavities were prepared on both buccal and lingual surfaces of each tooth using a fissure carbide bur (No 56) in a high speed hand-piece with air and water spraying to the following dimensions:

Mesiodistal length: 3 mm, cavity depth: 2 mm and cavity occlusogingival width: 2 mm to place the cavity at 1 mm occlusal to the CEJ (enamel margin), 1 mm gingival to the CEJ (dentin margin). The cavity dimensions were controlled using periodontal probe (Hu-Friedy Co. USA). The enamel margins of all cavities were beveled with fine-grit bur, while the dentin margins on the root surface were not beveled. The bur was replaced after preparation of 4 cavities. The prepared teeth were rinsed in tap water and stored in distilled water until restored.

Restoration placement

During the experiments, the teeth were rinsed and dried. Then, phosphoric acid, (Ultra-Etch 35%, Ultradent, USA) was used on the enamel and then on dentin surfaces, so that these surfaces were etch for 30 and 15 seconds, respectively. All surfaces were rinsed for 20 seconds with air-water spray. The excess water was removed by gentle air pressure. To avoid excessive drying of dentin surfaces, a semi-moist cotton ball was used on dentin surfaces after drying. The bonding agent (Single bond plus, 3M, ESPE, USA) was applied for all surfaces of cavities and the rubbing was performed with the same brush for 10 seconds and air-thinned gently with air stream, then the second bonding layer was applied and light-cured with an intensity of 450 MW /cm^2 for 20 seconds using light-curing device (Coltolux 75, Whaledent Inc, Coltene, USA). The intensity was adjusted using a radiometric device (Coltene, Whaledent Inc, USA). Then, the cavity was resorted horizontally in two increments with shade A3 Z100 composite resin (3M, ESPE, USA). The curing time of each layer was 40 seconds with the intensity of 450 MW/cm^2.The excess composite was excised with scalpel. All restored teeth were stored in distilled water at 37 ° C and kept until the beginning of the experiment.

Immersion of the samples in the treatment solutions

The restored teeth were randomly divided into four groups. Since the cavities were prepared in both buccal and lingual surfaces, each group consisted of 14 cavities of Class V. The experimental groups were distilled water (control group), 0.2% chlorhexidine, Oral B and Irsha mouthwashes. The pH values of the mouthwashes were determined by a PH meter (Table 1). The restored teeth were stored in 20ml of the test solution contained within a packed plastic bottle for 12 hours, which was reported as the equivalent time to 1 year using of mouthwash as 2 minute daily[11]. Therefore, the samples of each group were placed in solution for six hours for two consecutive days. The bottles were shaken every 1hour for 30 seconds to provide homogeneity. After 6 hours, the samples were removed, and rinsed for one minute, then they stored in 20 ml distilled water for 18 hours. The same procedure was repeated on the second day. The teeth were kept at 37 ° C throughout the two days/for two complete days. The samples were thermocycled between 55±2 ° C and 5±2 ° C (dwell time of 30 seconds) for 500 cycles following 24 hrs placement in distilled water.

Staining

The root apices of the teeth were sealed with sticky wax. All teeth surfaces were coated with two layers of nail varnish within approximately 1 mm of the margin of the restoration. The teeth were then immersed in 2% basic Fuschin solution at 37 ° C for 48 hours.

Sectioning and measurement of Micro leakage

The teeth were removed from the dye, rinsed in tap water and dried for two minutes, then mounted in epoxy resin (Epofix, EMS, and Fort Washington, PA, USA). The mounted samples were sectioned buccolingually through the center of the tooth using a diamond disk (Diamat, Germany) with a thickness of 0.5 mm. The sectioned specimens were examined under a microscope at 25x magnification.

The degree of micro leakage at occlusal and gingival margins was graded according to the following scale[22]:

0: No dye penetration

1: Dye penetration up to less than half the cavity depth

2: Dye penetration more than the half cavity depth, without axial wall involvement

3: Dye penetration along the axial wall

Data Analysis

Mean ranking and distribution frequency of the microleakage scores were calculated for each margin (occlusal and gingival) of each group. The data were analyzed using SPSS 19 program. The occlusal and gingival margins of four solutions were compared using the statistical test of Kruskal Wallis. The solutions as well as the occlusal and gingival margins of each solution were compared in pairs by using the statistical analysis of Mann Whitney test at a significance level of 0.05.

Table 1: mouthwashes which are used in this study

Mouthwash	Producer company/country	Components	PH
Oral B	Gillet, brazil	Sodium florid, glycerin, cetil perineum chloride, essence, sodium saccharin, methyl, paraben	5.95
Irsha	Shafa, Iran	Ochaliptole, methyl salicilate, benzoic acid, sodium saccharin, timol, ethanol	4.05
Chlorhexidine glococnate	Daroo Pakhsh, Iran	Chlorohexidine glochonat,cytramid,lidokaein,chloridric acid, essence, water	6.32

Results:

The results revealed that - at the occlusal margin - the highest (Mean Rank = 30.07) and lowest (Mean Rank = 25.36) dye penetration was corresponding to teeth that were immersed in the chlorhexidine mouthwash and distilled water, respectively. The Kruskal-Wallis test showed that this difference was not significant statistically (P = 0.777) (Table 2). Also the results revealed that, at the gingival margin, the highest (Mean Rank =35.1) and lowest (Mean Rank =25.18) dye penetration was corresponding to teeth that were immersed in the Oral B mouthwash and distilled water, respectively (Table 3). The Kruskal-Wallis test showed that there was no significant difference statistically (P = 0.232) at the gingival margin (Table 3). The Mann-Whietny test showed that the differences between the experimental groups were not significant statistically in both margins (P>0.05) (Tables 4, 5).

The Mean Rank difference of dye penetration between occlusal and gingival margins of the each group was not statistically significant (P <0.05) (Table 6).

Table 2. Comparisons of frequency and Mean Rank of the dye penetration depth among the experimental groups at the occlusal surface

penetration / Solution	Score 0		Score 1		Score 2		Score 3		total		Mean Ranks	P-value
	Frequency	%	frequency	%	frequency	%	frequency	%	frequency	%		
Distilled water	11	78.6	3	21.4	0	0	0	0	14	100	25.36	0.777
Oral B	10	71.4	1	7.1	2	14.3	1	7.1	14	100	29.07	
Irsha	9	64.3	4	28.6	1	7.1	0	0	14	100	29.50	
Chlorhexidine	9	64/3	3	21.4	2	14.3	0	0	14	100	30.07	

Table 3. Comparisons of frequency and Mean Rank of the dye penetration depth among experimental groups at the gingival surface

penetration / Solution	Score 0		Score 1		Score 2		Score 3		total		Mean Ranks	P-value
	Frequency	%	frequency	%	frequency	%	frequency	%	frequency	%		
Distilled water	9	64.3	2	14.3	2	14.3	1	7.1	14	100	25.18	0.232
Oral B	4	28.6	3	21.4	6	42.9	1	7.1	14	100	35.1	
Irsha	9	64.3	1	7.1	2	14.3	2	14.3	14	100	26.57	
Chlorhexidine	7	50	5	35.7	2	14.3	0	0	14	100	26.64	

Table 4. Comparisons of differences among the experimental groups at the occlusal surface

	Distilled water	Oral B	Irsha
Distilled water			
Oral B	P=0.486		
Irsha	P=0.366	P=0.956	
Chlorhexidine	P=0.324	P=0.889	P=0.892

Table 5. Comparisons of differences among the experimental groups at the gingival surface

	Distilled water	Oral B	Irsha
Distilled water			
Oral B	P=0.081		
Irsha	P=0.851	P=0.176	
Chlorhexidine	P=0.699	P=0.076	P=0.878

Table 6: Comparison of frequency and Mean Rank of color penetration between two surfaces of occusal and gingival in different groups.

Penetration / Solution / surface		Score 0		Score 1		Score 2		Score 3		total		Mean Ranks	P-value
		Frequency	%	frequency	%	frequency	%	frequency	%	frequency	%		
Distilled water	occlusal	11	78.6	3	21.4	0	0	0	0	14	100	13.18	P=0.284
	gingival	9	64.3	2	14.3	2	14.3	1	7.1	14	100	15.82	
OralB	occlusal	10	71.4	1	7.1	2	14.3	1	7.1	14	100	11.71	P=0.052
	gingival	4	28.6	3	21.4	6	42.9	1	7.1	14	100	17.29	
Irsha	occlusal	9	64.3	4	28.6	1	7.1	0	0	14	100	13.89	P=0.647
	gingival	9	64.3	1	7.1	2	14.3	2	14.3	14	100	15.11	
Chlorhexidine	occlusal	9	64.3	3	21.4	2	14.3	0	0	14	100	13.64	P=0.535
	gingival	7	50	5	35.7	2	14.3	0	0	14	100	15.36	

Discussion:

Nowadays, the use of plaque control antimicrobial mouthwashes and a variety of tooth-colored materials such as composite resin have been increased[1]. The strength and durability of adhesive bonds depend on several factors. These include the physicochemical properties of the adherend and adhesive materials; the structural properties of the adherent, which is heterogeneous; the formation of surface contaminants during cavity preparation; the development of external stresses that counteract the process of bonding and their compensation mechanisms; and the mechanism of transmission and distribution of applied loads through the bonded joint. Furthermore, the oral environment, which is subject to moisture, physical stresses, changes in temperature and pH, dietary components, and chewing habits, considerably influences adhesive interactions between materials and tooth tissues.The in vitro micro leakage tests offer valuable information about the sealing ability of adhesive resins[24].

This study revealed that micro leakage was minimal in distilled water group and maximal in occlusal and gingival margins of Chlorhexidine and Oral B groups, respectively, but this difference was not significant statistically. The diversity of the results was due to the differences in the factors that affect the surface roughness, hardness and micro leakage of restorative materials[25].

Some studies suggested that ethanol may lead to softening of restorative materials including composite resin[8-10]. Yanikoglu[16] and Gurdal et al[19]. argued that there is little difference between the effects of alcohol-containing mouthwashes and distilled water on the micro hardness of the composite, which is similar to our results. Unlike the present study, Penugonda[26] and Lavvaf et al[9] suggested that the alcohol-containing mouthwashes could affect the composite microhardness, but the softening effect depends on the alcohol content.The effect is more pronounced when resin polymerization is incomplete[8].

Gurgan et al[11]. found that both alcohol-containing and alcohol free mouthwashes influence the composite hardness, while our results have rejected the effect of alcohol on the micro leakage of Z_{100} composite. It should be noted that the aforementioned studies have investigated the effect of mouthwashes on the hardness of various composites, while our complementary research investigated the micro leakage, because other factors affect the micro leakage, in addition to the factors affecting the composite hardness.

Awliya[21] found that the micro leakage of vitalessence composite in alcoholic and alcohol-free mouthwashes was significantly more than in distilled water. In addition to the alcohol content, the pH difference and the fluoride concentrations of the mouthwashes lead to increase of micro leakage. Cavalcanti et al[27] confirmed that the APF gels that contain phosphoric acid lead to degradation of restorative materials. Although the mouthwashes used in the present study contained no fluoride, except Oral B which can play a role on the slight increase of micro leakage at gingival margins of the samples immersed in Oral B. Diab et al[10], suggested that the mouthwashes with low pH lead to greatest damage in restorative materials.

Gurdal et al[19]. and Celik et al[1] also showed that mouthwashes with various pH and alcohol content have no effect on microhardness and color of restorative materials, but the immersion time and difference of the sample preparation methods are more effective, which is similar to our results. Although, according to this study, chlorhexidine (pH = 6.32) and Oral B (pH =5.95) had higher pH than Irsha (pH =4.05), but no significant difference of micro leakage was observed. One of the main reasons for the diversity of results was different immersion times in solutions. In the Awliyas study, the immersion time was 24 hrs (equivalent to 4 minutes per day during a year); while in the current study the immersion time was 12-hour in two consecutive days for 6 hrs which is equivalent to 2 minutes per day during a year. The samples were placed in distilled water after each 6 hrs. Lavvaf et al[9] immersed the samples for 12 hrs without any interruption during a day. The immersion time in the test solutions affects the properties of restorative materials. Longer immersion time will lead to more damaging effects of ethanol[19].

The other compounds of mouthwashes may also make polymer matrix softening[11]. The Oral B contains Cetyl Pyridine Chloride (CPC). CPC is a cationic surfactant which can reduce surface tension of the liquid which leads to increase the wetting and penetration coefficient in capillaries. Also, chlorhexidine can influence in the bonding process due to adsorption of the tooth surfaces[22] which may be the cause of slight increase in micro leakage due to Chlorhexidine in occlusal margins and Oral B in gingival margins in the present study.

In another study, Awliya[28] and Yap et al[18]. found that the mouthwash type, immersion time and the type of restorative material may affect the cosmetic restorative materials. These studies showed that, Listerine adversely affected the hardness of composite resin, cemented glassinomer(GI) and resin modified glassinomer(RMGI). The Oracept had only affected the RMGI and GI. Although, Listerine contained high percentage of alcohol, but it had no effect on compomer[18,28].

The factors affect the polymerization shrinkage of resin composite including C-Factor, cavity size, the method for placement of composites, curing method and mechanical properties of the composite are among other factors lead to diversity of results[29]. The polymerization stress reducing principles were highly respected in the present study.

Also, incremental placement of the composite decreases the cured bulk[30] and C-Factor (The ratio of bonded to non-bonded surfaces)[31] similar to this study. The composites with lower matrix, higher filler content and larger particles will have less shrinkage[32,33]. The Z_{100} composite used in the current study consists of 85 wt% (66 vol%) 0.01-3.5 microns particles which was probably one of the reasons for lower polymerization shrinkage[34]. According to the elastic bonding concept theory, a thick elastic bonding resin is able to reduce the shrinkage stresses in the dentin bonding(stress breaker layer)[35], as Crim also found a reduction in micro leakage[36]. Two bonding layers have been used in this study.

Ajami et al[22] showed that the micro leakage of composite resin was influenced by the type of the adhesive system, mouthwash and location of the cavity margin. Since the micro leakage was indistinguishable among three mouthwashes of Clearfil SE adhesive group, the results were consistent with the results of present study, even though CSE is self-etched. But in the Excite adhesive group, the maximum micro leakage was observed for Oral B which was not in agreement with our results. The Etch and rinse adhesive systems are known as standard and some researchers have reported lower micro leakage compared with self-etched ones[37]. Giachetti et al[38] found similar micro leakage for both adhesive systems in the enamel and dentin margins. Several studies showed that the efficacy of bonding system is more dependent on operator or application protocol rather than chemical composition and generation classification[39,40]. The use of new adhesive systems with higher marginal adaptation and bond strength[41] as well as bonding with filler particles demonstrated greater clinical success[42].

As in the current study, the Single Bond plus bonding was used that is a fifth-generation bonding containing silica nanoparticles. Ajami et al[22]. bleached the samples with caramide peroxide prior immersion in mouthwashes. According to some studies, carbamide peroxide has detrimental effects on the bond strength of the composite resin to enamel, dentin and marginal seal[43-45]. Ayad et al[34]. found that, the micro leakage was dependent on the bleach concentration and the composition of composites. Therefore, it is likely that the micro leakage of composite was influenced by the bleaching material prior immersion in solutions. Also, as mentioned before, different types of bonding and composites could also be involved in the diversity of the results.

The pairwise comparison of the micro leakage of the occlusal and gingival margins of each group indicated that the micro leakage at the gingival margins of all groups was higher than occlusal surfaces, but the difference was not significant. These results are consistent with some studies[46,47], but they are not in agreement with some other studies and Ajami et al[22,48-50]. Awliya et al[21] found that the observed difference between the occlusal and gingival surfaces was not significant in distilled water and alcoholic mouthwashes with low pH, but it was significant for Plax and Emoform-F mouthwashes.

These differences may be due to the same factors as in the shrinkage during polymerization. Cenci et al[34] showed that the new bonding systems would have almost the same enamel and dentin bond values, so that the bonding and composite type of the present study was similar with those of latter study. Although the thermo-cycling method lead to thermal variations which is close to the clinical situation, but the effect of mouthwashes on restorative materials may vary in clinical condition due to several factors which may not be verified in vitro. Saliva may affect the pH of oral environment and neutralize or dilute the effects of the mouthwash[22].

As the conclusion of the present and other studies, it can be said that the type and composition of immersion solutions including the alcohol content, pH and other compounds, immersion time in solutions, the maintaining condition in the air or humidity of test environment, differences and diversity of the sample preparation methods, the diversity of bonding and composite materials, instruction for each bonding type, and effective factors for the shrinkage of composites during polymerization, especially the last one has a great impact on the micro leakage of composite materials.

Conclusions

Based on results of the present study, it revealed that the micro leakage was not dependent on the type of mouthwash and dental margins (enamel or dentin).

Given the results of this study and other studies, it is recommended to investigate the different factors affecting various properties of the restorative materials in the conditions close to clinical conditions, e.g. the use of artificial saliva.

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