

The impact of oxidative mouthwash on microleakage of composite restorations: A stereomicroscopic study

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Abstract

Objective: Although oxidative mouthwashes have many antimicrobial benefits, it has been suggested that residual oxygen interferes with composite resin adhesion to dental structures. This study aimed to evaluate the effect of an oxidative mouthwash on the microleakage of composite restorations.

Methods: Twenty-four extracted human third molars were randomly assigned into three groups: Group 1: a 0.05% sodium fluoride mouthwash, Group 2: an oxidative mouthwash, and Group 3: distilled water. The teeth were immersed in the corresponding solution for 10 minutes a day over 14 days. Class V cavities were prepared in the buccal and lingual surfaces of the teeth (n=15 per group) and restored with Filtek Z250 composite. The teeth were thermocycled between 5° C and 55° C for 1000 cycles, then immersed in 2% fuchsin solution for 24 hours, followed by sectioning in the bucco-lingual direction. The gingival and occlusal microleakage were inspected using a stereomicroscope. Data were analyzed using Kruskal-Wallis and Mann-Whitney U tests. The statistical significance level was considered at $P < 0.05$.

Results: The highest and lowest average microleakage scores were observed at the gingival and occlusal margin of cavities immersed in the sodium fluoride mouthwash, respectively. No statistical differences were observed in microleakage among the three groups either at the occlusal or at the gingival margin ($P > 0.05$). The Mann-Whitney U test showed a statistically greater microleakage at the cervical (1.05 ± 1.1) compared to the occlusal (0.694 ± 0.53) margins, irrespective of the treatment groups ($P = 0.033$).

Conclusions: Using an oxidative mouthwash does not affect the microleakage of composite restorations.

Keywords: Composite resins, Dental restoration, Microleakage, Mouthwash, Stereomicroscope

Introduction

Mouthwashes are integral to oral hygiene regimens and complement the effects of brushing and flossing. Mouthwashes possess antiseptic properties that significantly diminish the microbial load in the oral cavity. These solutions offer a spectrum of benefits such as declining halitosis, preventing periodontitis, and arresting early carious lesions (1). In light of the recent COVID-19 pandemic and the threat of other viral infections, there has been a renewed interest in the antimicrobial potential of mouthwashes.

Preliminary studies indicate that oxidative mouthwashes, notably those including hydrogen peroxide, might play a role in reducing the oral viral load, thereby proposing a potential to decrease the transmission risk of diseases (2). Some studies have shown that hydrogen peroxide at a concentration of 0.5% has a lethal effect on the envelope of viruses, including coronavirus (3).

The benefits of hydrogen peroxide extend beyond antiviral properties. Many individuals encounter challenges with tooth discoloration. Tooth discoloration is associated with different etiologic factors. Both extrinsic sources such as dietary stains, and intrinsic factors including medications, trauma, or even genetic predisposition can contribute to this condition (4). Oxidative mouthwashes, with their hydrogen peroxide content, serve as potent oxidizing agents, targeting and breaking down the chromogenic compounds responsible for enamel staining (5).

However, the presence of residual peroxide on the tooth surface may interfere with the bonding process and prevent the complete polymerization of the composite resin. Hydrogen peroxide may also alter the mineral and protein content of the enamel surface layer, leading to reduction in the bond strength (6).

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Oxidative mouthwash and composite resin microleakage

Microleakage is one of the major reasons for the failure of composite resin restorations. Penetration of bacteria and bacterial toxins into the gap between the restoration and the tooth can lead to secondary decay and pulpal irritation (7), eventually causing tooth hypersensitivity, marginal staining, and restoration failure (8). The dye penetration technique has been recognized as the most common method for evaluating the degree of microleakage. In this procedure, dye is applied to the restored tooth, and the penetration depth is measured and recorded at the interface of tooth and restoration (9).

Although numerous studies have evaluated the effect of different mouthwashes and bleach materials on the microleakage of restorations, few studies have investigated the impact of oxidative mouthwashes on the microleakage of composite restorations (10). Therefore, the present study aimed to examine the microleakage of gingival and occlusal margins of composite restorations in teeth exposed to an oxidative mouthwash, and compares it with a sodium fluoride mouthwash and distilled water. The null hypotheses of this study were that microleakage in teeth subjected to the oxidative mouthwash would not be different from teeth exposed to sodium fluoride mouthwash or distilled water, and microleakage would be similar at the gingival margins of the restorations compared to the occlusal margins.

Materials and Methods

Sample preparation

The protocol of the present *in vitro* study was approved by the Research and Ethics Committee of Shahid Sadoughi University of Medical Sciences (IR.SSU.REC.1400.061).

The present study was conducted on freshly extracted human third molars. The teeth were discarded if any cracks, caries, or defects were noticed. A total of 24 teeth were obtained. First, the teeth were cleaned with an ultrasonic scaler and then disinfected in a 0.5% chloramine T solution for 24 hours. Specimens were randomly divided into three groups of 8, according to the type of storage solution:

Group 1: a 0.05% sodium fluoride mouthwash (Oral-B fluoride mouthwash, USA)

Group 2: an oxidative mouthwash (Payadental, Iran) containing 0.1% hydrogen peroxide

Group 3: distilled water

Intervention

The teeth in the study groups were immersed in the respective solution for 10 minutes, rinsed with water, and then kept in distilled water until the next day. Storage solutions were changed daily and this procedure was repeated for 14 days. The teeth were finally removed from distilled water and dried. In each of the buccal and lingual surfaces of the teeth, an oval class V cavity was prepared with the following dimensions: mesiodistal width = 5 mm, occlusogingival height = 4mm, and axial depth = 2mm. Cavities were prepared by a high-speed handpiece with a diamond fissure bur (Tizkavan, Iran). The cavity was designed to include both enamel and cementum structures. Since 15 specimens were required for each group (n = 15), cavities were prepared on both the buccal and lingual surfaces of seven teeth and one tooth received only one buccal class V cavity preparation.

To restore the teeth, the enamel margins of the cavity were first etched with 37% phosphoric acid (Marvaben, Iran) for 20 seconds and then washed with water for 10 seconds. Then, a bonding agent (All-Bond universal BISCO, USA) was applied according to the manufacturer's instructions. The cavities were restored using four layers of Filtek Z250 composite (3M ESPE, USA) and then each layer was light cured with a LED curing device (Dentamerica, Taiwan) for 40 seconds at 800 mW/cm² intensity. The restored surface was finished and polished using Sof-Lex disks (3M ESPE, USA). Table 1 summarizes the characteristics of the materials used in the present study. The prepared samples were subjected to 1000 thermal cycles between 5°C and 55 °C with a dwell time of 30 seconds in a thermocycling device (TC-300, Iran).

Microleakage assessment

To examine the microleakage, the tooth surfaces were covered with two layers of nail polish up to 1 mm from the outer surface of the cavity margins. The apexes of the teeth were covered with wax to ensure that dye would only penetrate through the restoration margins. The samples were then immersed in a 2% fuchsin solution for 24 hours.

Specimen preparation and microleakage assessment was completed similar to the method described by Agrawal et al. (15). The specimens were cut buccolingually, by a Mecatome (T201, France) cutting machine. Dye penetration into occlusal and gingival margins was examined under a Leica stereomicroscope (Wetzlar, Germany) with 16 X magnification and graded as follows:

Grade 0: no color penetration.

Table 1: The characteristics of the utilized materials

Type of material	Commercial name	Company	Country	Compounds
Bonding agent	All-Bond Universal	BISCO	USA	Benzalkonium Chloride, 10-MDP (Phosphoric acid ester monomer), Bis-GMA, HEMA, Ethanol, Water, Initiators
Composite resin	Z250	3M ESPE	USA	Water, Glycerin, Cetylpyridinium Chloride, Flavoring, Bis-GMA, UDMA, Bis-EMA
Fluoride mouthwash	Oral B fluoride mouthwash	Oral-B Laboratories	USA	Sodium fluoride, Sodium Saccharin, Methylparaben, Phosphoric acid, Sucralose, Poloxamer, Propylparaben, Disodium Phosphate
Oxidative mouthwash	Payadental	Irox	Iran	Zinc, Sodium lauryl ether sulfate, Sodium lauryl sulfate, Hydrogen peroxide, Polysorbate, Glycerin, Sorbitol, Fluoride, Potassium nitrate, Herbal flavoring, Water

Grade 1: The dye penetration depth was less than 1/2 of the cavity depth.

Grade 2: The dye penetration depth was greater than 1/2 of the cavity depth and without penetrating the axial wall.

Grade 3: Dye penetration was observed in the axial wall.

Sample size calculation and statistical analysis

Data were subjected to statistical analysis using SPSS, version 25 (IBM Corp, Armonk, NY, USA). Based on the results from Ozduman et al. (10), a minimum sample size of 15 cavities was determined for each study group, with 45 samples in three groups, taking into account the 95% confidence level and power of 80%.

The frequency distribution of microleakage scores according to groups and margins was investigated using the Kruskal-Wallis and Wilcoxon signed-rank tests, respectively. A statistical significance level of 0.05 was considered in all of the tests.

Results

Table 2: Mean, standard deviation (SD), and median microleakage scores according to the study groups and restoration margins

group	Occlusal		Gingival	
	Mean \pm SD	Median	Mean \pm SD	Median
Oxidative mouthwash	0.640 \pm 0.47	0	0.834 \pm 0.87	1
Fluoride mouthwash	0.632 \pm 0.60	1	1.234 \pm 1.33	1
Distilled water	0.834 \pm 0.53	0	0.915 \pm 1.13	1
P-Value	0.962		0.991	

The greatest and least average microleakage scores were observed at the gingival and occlusal margin of cavities immersed in the sodium fluoride mouthwash, respectively (1.23 \pm 1.33 and 0.632 \pm 0.60). The Kruskal-Wallis test showed no significant difference in the mean microleakage scores between the three groups either at the occlusal (P= 0.962) or at the gingival (0.991) margin (Table 2).

The mean overall microleakage score for the gingival margin (1.05 \pm 1.11) was significantly higher than that of the occlusal (0.69 \pm 0.53) margin (P = 0.033), irrespective of the group type (Table 3).

Discussion

The results of the present study showed no significant differences between the microleakage of the oxidative mouthwash group and the two other groups, which confirms the first hypothesis of the study. However, the microleakage at the gingival margin was significantly greater than that of the occlusal margin (P=0.033), thus rejecting the second hypothesis.

Table 3: Total Mean, standard deviation (SD), and median microleakage scores according to the study groups

group	Occlusal			Gingival			P-value
	n	Mean±SD	Median	Mean±SD	n	Median	
microleakage scores	45	0.69 ± 0.53	0	1.05 ± 1.11	45	1	0.033

In vitro, microleakage tests provide valuable information on the ability of restorative materials to seal cavity margins. The most common technique for microleakage evaluation is the dye penetration test (16, 17), which was used here for the evaluation of microleakage at the enamel and dentin margins of Class V cavities.

The microleakage of composite restorations is generally related to mechanical stress due to polymerization shrinkage. Factors that affect the amount of stress due to polymerization shrinkage include the cavity configuration factor (C-factor), i.e. the ratio of the bonded surface area in a cavity to the unbonded surface area, cavity size, the mode of application of the light-curing device, and the mechanical properties of the composite resin (16). Therefore, only one type of composite, and light-curing device and method was used in this study and the shape and size of all prepared cavities were similar. To simulate the oral environment; all samples underwent thermocycling.

In the present study, the highest amount of microleakage belonged to sodium fluoride mouthwash. However, no significant differences were observed in the amount of microleakage between the three groups.

Although many studies have investigated the effects of different mouthwashes on the microleakage of restorations, few studies have examined the effect of oxidative mouthwashes on the microleakage of composite restorations. Ajami et al. (18) investigated the effect of three different types of mouthwashes on the microleakage of composite resin restorations after bleaching with 10% carbamide peroxide. Their results showed that the microleakage of mouthwashes containing fluoride and carbamide peroxide was not significantly different, which is consistent with the present results. An in vitro study by Ozduman et al. (10), evaluated the effect of SARS-COV-2 antiviral mouthwash on the bond strength of a universal adhesive to enamel. The authors reported that the mouthwash containing hydrogen peroxide reduced the shear bond strength of the adhesive, which was in contrast to the results of the present study. This may be attributed to the

use of different types of bonding systems or the different concentrations of hydrogen peroxide between studies. On the other hand, the teeth were exposed to the mouthwash before restoration in the present study, as opposed to tooth exposure to the mouthwash after restoration in the study of Ozduman et al (10). It is also possible that hydrogen peroxide mouthwash decreases SBS for composite but does not render the restorations more prone to microleakage.

Other research on the influence of hydrogen peroxide on the bond strength and microleakage of composite restorations used a variety of bleaching materials. In a review article, Alqahtani et al. (6) concluded that the presence of peroxide residue on the tooth surface could reduce the bond strength of composite restoration to the tooth. Many studies examined the effect of bleaching after restoration and found that hydrogen peroxide, especially at high concentrations, could affect the bond strength or microleakage of old restorations (18-20).

In the present study, the oxidative mouthwash was used for two weeks before restoration. In the in vitro study of Barbosa et al. (21), a decrease in the bond strength was present in dentin after 14 days of hydrogen peroxide bleaching treatment, and this decrease in the bond strength was still significant after 70 days. They attributed this long-term effect of bleaching to the structural characteristics of dentin which in contrast to enamel, can serve as a reservoir for bleaching materials. The remained bleaching materials sometimes decompose into oxygen and water. Oxygen release can both interfere with resin entry into etched dentin and hinder resin polymerization (22).

It should be noted that the concentration of hydrogen peroxide used in the mouthwash is different from that of bleaching agents. The mouthwash used in the present study contains a concentration of 0.1% hydrogen peroxide, which is far from the concentration used in bleaching materials. Furthermore, the time of application of the solution or mouthwash containing hydrogen peroxide or the type of prepared cavity, the bonding system applied, as well as the number of samples and

laboratory procedures, vary in different studies. Therefore, it is not possible to accurately compare the results of the present study with previous research.

Bistey et al. (23) evaluated changes in the human enamel by Fourier transform infrared (FTIR) spectroscopy after treatment with hydrogen peroxide. They found that exposure to hydrogen peroxide for more than 30 min resulted in a significant reduction in phosphate ions. Therefore, the addition of phosphoric acid can reduce the negative effect resulting from residual oxygen on bond strength. Using phosphoric acid treatment after hydrogen peroxide has been suggested to increase the bond strength (10, 19). Therefore, in the present study, selective enamel etching was used before applying universal bonding. The reason for the lack of microleakage differences between the three study groups may be related to the use of phosphoric acid.

Another result of the present study was that the microleakage score in the gingival margin was significantly higher than that in the occlusal margin regardless of the type of mouthwash. This result rejects the second hypothesis of this study. The outcomes of this study are consistent with those of Goyal et al. (20) and Ajami et al. (18), who showed that the amount of microleakage was higher in the gingival margin. This is because the enamel structure is highly mineralized, but dentin contains a large amount of water and organic substances, which makes bonding to dentin more difficult.

Cagidiaco et al. (24) attributed dentin marginal leakage to the absence of dentin tubules over a distance of 100 µm from the cervical margin toward the axial wall in the gingival floor of the proximal box. Osorio et al. (25) showed that the enamel was thin in the cervical margin and had little tendency to bond with the adhesive. As a result, the resin composite establishes a stronger bond with the occlusal margin, and more microleakage occurs in the gingival margin.

In this *in vitro* study, it was not possible to simulate all clinical conditions. It seems that the presence of zinc in the oxidative mouthwash might have affected the microleakage in the samples and prevented the negative effect of hydrogen peroxide. Furthermore, microleakage was evaluated by the dye penetration technique in this study. A disadvantage of this method is the semi-quantitative information and random selection of the cutting axis, which may not represent the place where the greatest depth of color penetration occurs (26). Clinical examination or the use of electron microscopy is recommended in future studies to better evaluate the amount of microleakage.

Conclusions

There were no significant differences between the mean microleakage scores of composite restorations in the oxidative mouthwash, fluoride mouthwash, and distilled water groups. Regardless of the utilized mouthwash, the amount of microleakage in the gingival margin was significantly higher than that in the occlusal margin.

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