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Research Paper

Antioxidants Reduce Microleakage of Resin-based Composite Fillings and the Associated Toxicity After Bleaching Human Teeth

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ABSTRACT

Background: Bleaching teeth is popular because the discoloration is a major people's concern. This study aimed to compare the effect of three antioxidants on the microleakage of composite resin filling after bleaching teeth with 35% H_2O_2, thus minimizing the potential clinical toxicity of filling materials entering patients' body.

Methods: Sixty-six extracted intact teeth were included in this study. After preparation of a ClV cavity on the buccal surface, the teeth were randomly divided into six groups (n=11, each). Except for group A (control), all others were bleached. Cavities in group A were filled with composite resin. In group B, the samples were immediately restored after bleaching while in group C, the filling was delayed for two weeks after bleaching. In groups D, E, and F, the cavities were treated with either sodium ascorbate, ascorbic acid, or vitamin C, and the filling restored. Teeth were sectioned and the microleakages examined microscopically, and the data were analyzed statistically.

Results: Group A showed the least amount of microleakage. In Group B, there was a significant increase in the microleakage when the samples were filled immediately after bleaching. The microleakages among groups of C, D, E, and F were similar to those in group A.

Conclusion: The microleakage increased significantly after bleaching with 35% H_2O_2, while treating dental cavities with the antioxidants effectively reduced the microleakage. Thus, delayed filling is a useful approach to minimize the microleakage. The findings help reduce or prevent the clinical toxicity arising from the microleakage of filling materials.

Keywords: Antioxidant, Bleaching, Composite resin, Microleakage, Toxicity

Introduction

Considering the improved public knowledge and cosmetic consciousness in the society, having white, well-aligned teeth is a symbol of health and beauty. For these reasons, bleaching teeth has become popular in the current decade [1]. Discoloration has become a major concern for people who care about the appearance of their teeth. Anteriorly discolored, deformed, or abnormally shaped teeth leads to significant aesthetic concerns in patients, often preventing them from smiling. Addressing this dental problem can significantly enhance the person's appearance, self-esteem, confidence and the quality of life [2]. Bleaching teeth with antioxidants, as a conservative method, has few adverse effects while improves the beauty in most patients, compared to direct restorations, ceramic veneers or enamel discoloration of teeth [3-7].

Previous studies have shown that bleaching teeth is relatively safer than making structural change to teeth. However, there are still public concerns about the negative effects of bleaching on the restorative materials and their adhesion to teeth and associated tissues [8-10]. Most patients undergoing teeth bleaching already have fillings and/or restoration materials in their teeth. The mechanisms for bleaching teeth involve chemical treatments with possible adverse effects on the physical and mechanical properties of both dental tissues and the restorative materials already in teeth [11]. The effects of bleaching agents on teeth and the restorative materials have been studied in recent years [12-14]. One of the major concerns is that the chemical softening caused by bleaching materials may affect the clinical durability of composite fillings in teeth [15].

Studies suggest that bleaching procedures may adversely affect the physical properties, color, and marginal integrity of restorative materials, and also the bonding of enamel and dentin [14, 16-18]. Bleaching materials increase the surface roughness of composite resins and reduce the microhardness of composite surface and teeth [19]. Microleakage due to frequent composite restorations before and after bleaching is another side effect of teeth bleaching materials. This is another major challenge to the success of all types of restorations [1, 20]. The remaining oxygen and peroxide on the teeth surface after bleaching is believed to be the reason for microleakage, which prevents the complete resin polymerization [13, 21-25]. Thus, different methods have been suggested to minimize these problems. For instance, to remove the surface enamel layer, we may use alcohol for bleached enamel before filling, or adhesive-containing organic solvents, or clean the cavity with a catalase, or use antioxidants, such as sodium ascorbate. Ascorbic acid, vitamin C, vitamin E and avoiding any filling from 24 hours to three weeks after bleaching, have been recommended [8, 25-30]. The effects of different antioxidants, such as vitamin C, ascorbic acid and sodium ascorbate have been studied before but their effects on microleakage have not been compared with each other. Most of the earlier studies assessed the microleakage from composite resins, with which the teeth were restored.

Aim of the study: The aim of this in vitro study was to assess and compare the effect of the three antioxidants on the microleakage of composite restorations that were used for filling after bleaching treatment. The microleakage after a 2-week delay between the restorative treatment and bleaching procedure was also evaluated. The findings of this study help minimize or prevent the potential toxicity caused by the microleakage of the dental filling materials in patients.

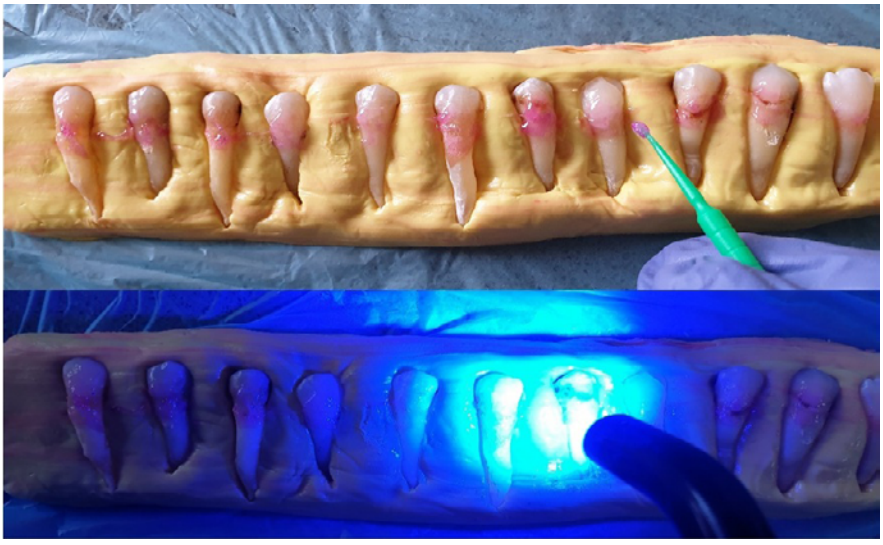
Material and Methods

Sample collection: Ninety maxillary and mandibular intact premolars extracted for orthodontic purpose or periodontal problem were collected during 10 months and stored in normal saline. Sixty-six teeth with intact buccal surface and without any crack, caries, and history of previous dental treatments were selected finally. The teeth were cleaned using a scalpel, polished with rubber cap and pumice paste, then randomly divided into six separate groups (n=11, each). A brief procedure has been outlined for each of the groups in Table 1.

Cavity preparation: A box-shaped ClV cavity with 4 mm mesiodistal in width, 2.5 mm in occlusogingival length, and a 1 mm in depth was produced on the buccal surface of each tooth, using a 1 mm diamond fissure bar (Z&D, Berlin, Germany) in a high-speed handpiece under air and water-cooling. After the cavity preparation, the gingival margin was identified at 1 mm apical to cemento-enamel junction (CEJ) on the root cement and the incision margin was on the tooth enamel. Each bur was used for only six teeth.

Bleaching procedure: The teeth were bleached, using FGM® bleaching material (Joinville, SC, Brazil) according to the manufacturer's instruction, then light-cured for 20 seconds with back-and-forth movements on 11 teeth (Figures 1a-b). The bleaching material was displaced on each tooth with an applicator every 5 minutes, and the teeth were rinsed for one minute after the 15-min treatment. This procedure was repeated three times.

Composite resin restoration: The enamel and dentin surfaces of all cavities were etched by 37% phosphoric acid (Morva Bon Co., Tehran, Iran) for 30 sec, then rinsed with water for another 30 sec., dried using cotton pellets, and spread gently with air. Following the thrush view on the buccal enamel surface, the bonding agent (single Bond, 3M Dental Products, St Paul, MN, USA) was applied with a micro-brush, and light-cured for 20 seconds, using LED curing device (Guilin Woodpecker Medical Instrument Co., China) at an intensity of 1200 mW/cm^2. This light intensity was regularly calibrated to ensure similar application for all specimen. Enamel A2 composite resin (Z350, Filtek Z350XT; 3M ESPE) was placed into cavities and polymerized in 1.5-2 mm thick layers for 40 seconds according to the manufacturer's instructions (Figures 2a & 2b). The composite filling was then polished, using a bur, the teeth were preserved at room temperature and distilled water, and were grouped as follows:



Figures 1. The bleaching process of the teeth specimens

● **Group A: (Control):** The cavity was prepped and filled with composite resin without a bleaching procedure.

● **Group B: (Immediate filling):** The buccal surfaces of the teeth were bleached, a box-shaped ClV cavity was made immediately after bleaching, then filled with composite resin as described earlier.

● **Group C: (Delayed filling):** The teeth were bleached as described above, then stored in distilled water at room temperature. After a 2-week period, cavities produced and were subsequently restored with composite resin.

● **Group D: (Sodium ascorbate):** The teeth were bleached and cavities prepared immediately after bleaching. The cavities were treated with 10% sodium ascorbate, by dissolving 10 mg of the powder (99% L-ascorbic acid sodium salt, Fluka, Switzerland) in 90 ml distilled water, using a micro-brush. The samples were rinsed after 10 min, dried with air, and restored with composite resin.

● **Group E: (Ascorbic acid):** The teeth were bleached and cavities prepared immediately after a bleaching process. The cavities were treated with 10% ascorbic acid solution, dissolved 10 mg of ascorbic acid powder (Merck, Darmstadt, Germany) in 90 ml of distilled water, using a micro brush, then rinsed after 10 min and

Figures 2. Box-shaped ClV cavity preparation and composite resin restoration

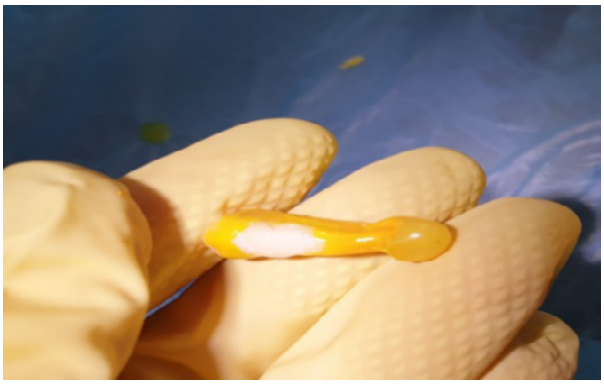


Figure 3. Covering the teeth surfaces with adhesive wax and nail polish to make a permeable barrier against the dye penetration

dried with air. Subsequently, the cavities were restored with composite resin as described above.

- **Group F: (Vitamin C):** The cavities were treated with a vitamin C solution for 10 min (500 mg/5 mL ascorbic acid, Daroo Pakhsh, Tehran, Iran), following bleaching and immediate cavity preparation. The teeth were then rinsed, dried and filled with composite resin.

Table 1 outlines a brief procedure for each of the six groups of teeth specimens. Finally, the teeth surfaces in all groups except the one with a 1 mm area around the margin of the restoration, were covered with three layers of nail polish (1 mm thick, each), and waxed to make an impermeable barrier against dye penetration. The apex of the teeth was also covered with adhesive wax (Figure 3). The teeth were immersed in 2% fuchsin and kept for 24 hours at room temperature. These samples were rinsed and dried at room temperature for 48 hours. They were longitudinally cut from the buccal to lingual surface along with the center of the fillings with a low-speed water-cooled thin sectioning diamond machine (Gillings-Ham Co., NY, USA). See Figure 4. These samples were evaluated under a stereo-microscope (Zeiss, Germany) at x40 magnification to examine their microleakage into the occlusal and gingival tissue margins (Figure 5). The results were checked by two independent examiners who were blinded to the study groupings. Next, each examiner reported the depth of the dye penetration based on the following scale:

Microleakage Evaluation Scale

- 0=No microleakage.
- 1=Microleakage penetrated to 1/2-depth of the cavity wall.
- 2=Microleakage penetrated beyond the 1/2-depth of the cavity wall.
- 3=The microleakage penetration reached the cavity bottom surface.

During the above evaluation process, the occlusal and gingival walls were scored separately.

Statistical analyses: The data were analyzed with the Kruskal–Wallis's test to evaluate the differences in the microleakage of the tested groups. Mann–Whitney statistical test was used to compare the groups in pairs. The Stata software, version 11 was used for the

Table 1. Brief procedure for different teeth groups

Group	Bleaching with 35% H_2O_2	Composite Resin Restoration Time	Antioxidant Treatment
A	None	Immediate	None
B	√	Immediate	None
C	√	After two weeks	None
D	√	Immediate	Sodium Ascorbate
E	√	Immediate	Ascorbic Acid
F	√	Immediate	Vitamin C

Figures 4. Buccolingual incision of the teeth with a low speed diamond saw

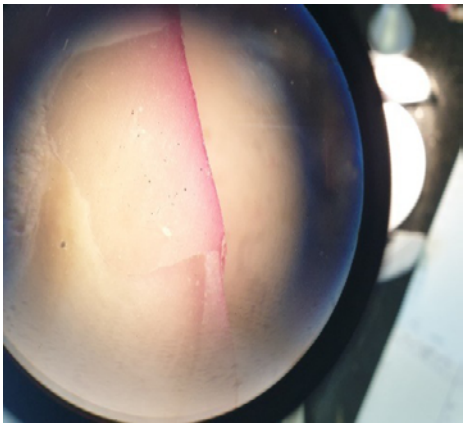


Figure 5. Dental image as viewed under a stereo microscope

purpose of data analysis. The statistical significance level was set at $P<0.05$.

Results

The results of the microleakage study in various tested teeth groups are presented in Table 2. Based on the study data, the highest amount of microleakage was observed in both the enamel and dentin in Group B where the cavities were restored immediately after bleaching. The lowest microleakage was observed in the restorative Group A, in which there was no tooth bleaching. There was a significant reduction in the microleakage in Group C, which had a 14-day delay

Table 2. Results of Microleakage in the study groups

Groups	Enamel			Dentin		
	Sum Rank	df	P*	Sum Rank	df	P*
Control Group	236.5	5	0.000	254.5	5	0.000
Immediate filling Group	639.0			626.0		
2-week delay restoration	291.5			281.0		
Sodium Ascorbate	335.0			379.0		
Ascorbic Acid	346.5			318.0		
Vitamin C	362.5			352.5		

* The significance level is considered to be 0.05 by Kruskall-Wallis test; df: degree of freedom.

between the bleaching process and the restoration with composite. The amount of microleakage in both enamel and dentin was approximately similar to that observed in the control group (P≥0.05). In groups D, E and F, a significant reduction occurred in the microleakage of composite resin fillings, the extent of which was not significantly different from that observed in Group C and the controls. Table 2 presents the results of microleakage in the study groups.

Discussion

Numerous studies have been conducted on the interactions of bleaching materials and the bonding strength of composite resin to the bleached teeth enamel [8, 25, 30-37]. These studies showed a significant reduction in the bonding strength of resin composite to the bleached enamel compared to the unbleached ones. However, there have been few studies conducted to date on the microleakage of composite resins into the gingival tissue or the patients' system after teeth bleaching [38, 39]. The results of the current study demonstrated a significantly higher increase in microleakage into the enamel and dentin layers in the groups that had immediate composite restoration following teeth bleaching than those observed in the non-bleached groups. Our findings were consistent with those reported by previous studies [37, 40].

Two previous studies [36, 41] have demonstrated negative effects of composite contact with 10-16% carbamide peroxide gel or 35% hydrogen peroxide, and enamel or dentin marginal seal. Further, Shinohara, et al. [42] have shown that immediate dental filling after bleaching of teeth has contributed to higher microleakage into dentin margins without affecting the enamel margins. This study's findings contrasted with our results, possibly because they used different bleaching materials and methods. Specifically, that study used a nonvital bleach technique with sodium perborate and 37% carbamide peroxide gel, as opposed to using vital bleaching method and 35% hydrogen peroxide in the current study. We found no significant differences in the enamel or dentin microleakage between the control group that received composite restoration without bleaching, and the group that had a 2-week delay before the teeth received fillings after bleaching.

A previous study conducted by Al-Hasani, et al. [40] has also emphasized that a 7-14-day delay for avoiding the negative effect of bleaching on the microleakage of filling materials. There was a significant reduction of the microleakage of three antioxidants (sodium ascorbate, ascorbic acid and vitamin C) found in this study, and the differences were not significant compared to the group that had a 2-week delay after bleaching or the controls. These results were consistent with those reported by previous studies on the assessment of antioxidant's effects on bleaching processes including higher bonding strength and lower microleakage potential [29, 40, 43-48]. Han, et al. [38] have demonstrated that sodium ascorbate treatment has not been able to change the extent of microleakage alone. They have suggested that this outcome should be achieved by using surfactant (Tween 80), which is not consistent with the current study findings. The inconsistency might be because of different treatment durations with ascorbic acid. The treatment period was 10 minutes in the current study as opposed to only one minute in the study conducted by Han, et al. [38]. The difference in the findings exist despite the fact that sodium carbonate solution was used at 10% concentration in both studies.

Of note, vitamin C was considered as being equivalent to ascorbic acid in previous studies; however, this vitamin has other components in addition to ascorbic acid. Also, an accurate comparison has not been made between sodium ascorbate and ascorbic acid in previous studies despite the similarities in their oxidative properties [27, 43, 49, 50]. In the current study, no significant differences were found among the three antioxidants (sodium ascorbate, ascorbic acid & vitamin C) in terms of the contribution of each compound to minimizing the microleakage of the composite resin. Considering the structure of composite bonding to enamel and dentin, it may be argued that higher microleakage of enamel may occur compared to that of dentin. This observation has been demonstrated in the current study, which was consistent with those reported by earlier studies [51, 52].

Conclusions

To prevent a significant microleakage in composite resin filling immediately after teeth bleaching, a two-week delay before a restoration treatment is suggested in order to reach the same microleakage level to non-bleached teeth due to the release of free radicals. However, in cases of no or shorter than a 2-week delay, the use of either of sodium ascorbate, ascorbic acid or vitamin C, is effective in minimizing or preventing the microleakage, which has similar results to a 2-week delay. In consideration of the same influence of the three antioxidants on microleakage, vitamin C is suggested because of being readily available than the other two antioxidants. We propose the evaluation of the effect

of other antioxidants on the microleakage of composite resin filling after teeth bleaching. Also, the influence of the tested antioxidants used in the current study on other bonding materials (e.g. self-etch) may be considered. The findings of this study help minimize or prevent the potential clinical toxicity to the patients' gingival tissue or systemically from the microleakage of the composite resin materials used for the filling of dental cavities.

Ethical Considerations

Compliance with ethical guidelines

The experimental protocol of this study was reviewed and approved by The Ethics Committee, Arak University of Medical Sciences, Arak, Iran (Registration Code: IR.ARAKMU.1398.170).

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Authors' contributions

All authors contributed fairly equally in conducting all aspects of this study. Also, they reviewed and approved the final draft of the manuscript.

Conflict of interest

The authors declare no conflict of interest with any internal or external entity in conducting this study.

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