



EFFECT OF OZONE PRETREATMENT ON THE MICROLEAKAGE OF CLASS V CAVITIES FOLLOWING OFFICE BLEACHING WITH DIODE LASER

Diyot Lazerle Yapılan Ofis Beyazlatma Tedavisi Sonrası Ozon Uygulamasının Sınıf V Kavitelerde Mikrosızıntı Üzerine Etkisi

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ABSTRACT

Objective: This study investigated the effect of ozone pre-treatment on the microleakage of class V composite restorations after using two different bleaching agents with laser activation.

Methods: Forty non-carious maxillary central incisor teeth extracted for periodontal disease were included and randomly divided into two main groups (n=20). *Group 1:* Bleaching agent including 40% H₂O₂ was activated with laser (Diode 980 nm, Gigaa Dental Laser Cheese, China). *Group 2:* Bleaching agent including 35% H₂O₂ was activated with the same laser. Then, the teeth were randomly divided into two subgroups (n=10). Ten specimens in each subgroup were subjected to ozone treatment (Ozonytron XP-OZ, MIO International, Germany) for 30 s, while the remaining untreated specimens were left as control group. Class V cavities were prepared on the buccal surfaces and restored with composite resin (Filtek Z550, 3M ESPE, USA). Following thermal cycling (5–55°C, 5000×), the specimens were kept in 0.5 % basic fuchsin for dye penetration within a period of 24h. Then, the teeth were sectioned longitudinally. The depth of staining along with the tooth-restoration interface was examine under a stereomicroscope and recorded.

Results: There were no significant differences between the two bleaching agents in terms of microleakage of restorations (p > 0.05). The ozone pre-treatment didn't decrease the microleakage values (p > 0.05). Significantly higher microleakage scores were determined at gingival margins compared to occlusal margins (p < 0.05).

Conclusion: Ozone pre-treatment did not affect microleakage scores of class V composite restorations following dental bleaching.

Keywords: Dental bleaching, diode laser, ozone, microleakage.

ÖZ

Amaç: Bu çalışmada, iki farklı beyazlatma ajanı kullanılarak yapılan lazer aktivasyonlu beyazlatma sonrası ozon uygulamasının, sınıf V kompozit restorasyonların mikrosızıntısı üzerindeki etkisi araştırılmıştır.

Metot: Çalışmada periodontal hastalık nedeniyle çekilen 40 tane çürüksüz maksiller santral diş kullanıldı ve rastgele iki ana gruba ayrıldı (n=20). *Grup 1:* %40 H₂O₂ içeren beyazlatma ajanı lazerle aktive edildi (Diode 980 nm, Gigaa Dental Laser Cheese, China) *Grup 2:* %35 H₂O₂ içeren beyazlatma ajanı aynı lazerle aktive edildi. Daha sonra dişler rastgele iki alt gruba ayrıldı (n=10). Her ana grubun, bir alt grubundaki 10 örnek kontrol grubu olarak ayrılırken, geri kalan 10 örneğe 30 saniye süreyle ozon (Ozonytron XP-OZ, MIO International, Germany) uygulandı. Bukkal yüzeylerde sınıf V kaviteler hazırlandı ve kompozit rezin (Filtek Z550, 3M ESPE, ABD) ile restore edildi. Termal siklattan (5-55 °C, 5000 ×) sonra örnekler, boyanın nüfuz etmesi için 24 saat boyunca %0,5 bazik fuksin içinde bekletildi. Ardından, değerlendirme için dişler vertikal olarak separe edildi. Diş ve restorasyonun arayüzü boyunca oluşan boyanma derinliği stereomikroskop altında incelendi ve kaydedildi.

Bulgular: Restorasyonların mikrosızıntısı açısından iki beyazlatma ajanı arasında anlamlı bir fark bulunamadı (p> 0,05). Ozon tedavisi mikrosızıntı değerlerini azaltmadı (p> 0,05). Okluzal marjinlerde, gingival marjinlere oranla anlamlı düzeyde daha fazla mikrosızıntı tespit edildi (p <0,05).

Sonuç: Beyazlatma sonrası ozon tedavisi sınıf V kompozit restorasyonların mikrosızıntı değerlerini etkilemedi.

Anahtar Kelimeler: Dental beyazlatma, diyet lazer, ozon, mikrosızıntı

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INTRODUCTION

Office bleaching is a conservative, effective and relatively safe esthetic treatment. In contemporary dental practice, agents containing 30-40 % hydrogen peroxide (H₂O₂) have been widely used for bleaching.¹ Although this chemical can be used for both home and office bleaching, the latter one has many advantages including professional control, avoiding the contact of agents with soft tissues, immediate results and bleaching of all surfaces of teeth.² H₂O₂ is a very unstable molecule and can be easily decomposed which results in the release of different oxygen species (e.g. perhydroxyl anion, hydroxyl radical). The amount of released oxygen radical depends on environmental conditions including pH, light, and temperature.³ For this reason, this agent can be used either alone or can be activated by heat or light in order to increase its efficiency.⁴

H₂O₂ may also alter the structure of both methacrylate-based composite resins and tooth including color, roughness and surface hardness. These changes may lead to decreased bond strength and increased microleakage throughout restorative material and dental hard tissue interface⁵ because these agents reduce polymerization if restoration carried out immediately after tooth bleaching.⁶ It is suggested that a period of time should be allowed between the end of bleaching treatment and placement of adhesive restorations. At least a period of 7 days is required for accomplishment of composite restoration immediately after bleaching.⁷

Today, the use of ozone in restorative dentistry has been used for pit and fissure caries and white spot lesion. Antibacterial property of ozone is due to high oxidation activity inducing the rupture of the cell membranes and destroying of intracellular components.⁸ The improving of equipment to utilize gaseous ozone in the dental practice has

allowed new therapeutic facilities in dental surface pre-treatment.⁹

Microleakage is one of the important factors that lead to the failure of resin restorations. It is defined as the penetration of bacteria, fluids, molecules or ions into the spaces between the cavity walls and the restorative materials.¹⁰ Clinically, microleakage can lead to postoperative sensitivity, staining around the margins of restorations, secondary caries, restoration failure, pulpal pathology or pulpal necrosis, partial or total loss of restoration.¹¹ It is known that there was a negative influence of bleaching on the marginal integrity of existing or new restorations.¹² On the current literature there have been many studies which related to the microleakage of composite resin restorations after tooth bleaching.^{13, 14} But, there is no available study about ozone treatment after bleaching in the literature. In the light of these informations, the aim of this study was to investigate effect of ozone application after office bleaching treatment on microleakage of class V composite resin restorations.

The null hypotheses to be investigated in this study are:

There are no differences between the microleakage values of different bleaching materials.

Ozone application doesn't affect the microleakage results of class V composite resin restorations.

MATERIALS AND METHODS

This study was approved by the ethical committee of Gaziantep University (2017/381). Forty non-carious maxillary central incisor teeth extracted for periodontal disease were included. External root surfaces of the teeth were cleaned with scalers to remove residual organic tissue. Then, the teeth

were randomly divided into two main groups (n=20).

Group 1: Bleaching agent including 40% H₂O₂ (Opalescence Boost, Ultradent Products) was activated with laser (980 nm Diode, Gigaa Dental Laser Cheese, China) for 3 cycles of 20 s with an output power of 4 w localized per teeth. The total bleaching procedure lasted 30 minutes totally.

Group 2: Bleaching agent including 35% H₂O₂ (Whiteness HP Blue, FGM Dental Products, Brazil) activated with laser (980 nm Diode, Gigaa Dental Laser Cheese, China) with the same procedure.

The teeth were further randomly divided into two subgroups (n=10);

Group 1a: Ozone pre-treatment following laser activated bleaching (40 % H₂O₂).

Group 1b: No ozone pre-treatment following laser activated bleaching (40 % H₂O₂).

Group 2a: Ozone pre-treatment following laser activated bleaching (35 % H₂O₂).

Group 2b: No ozone pre-treatment following laser activated bleaching (35 % H₂O₂).

Ozone was applied to external coronal surfaces of the teeth by using an ozone device (Ozonytron XP-OZ, MIO International, Germany) which has a plasma probe for caries treatment or prevention. The device was used with a concentration of 100 ppm for 30 seconds according to the manufacturer's instructions. Then, all specimens were immersed in distilled water and kept for 24 hours. Standardized class V cavities (2 mm depth, 4 mm mesiodistal, 2 mm occlusogingival) were prepared with cylindrical burs attached to aerator on the buccal surfaces in enamel over cement-enamel junction (CEJ) 24 hours later. Each cavity was etched for 30 s on enamel surface with 37% phosphoric acid (K-ETCHANT, Kuraray Noritake Dental Inc), then 40 s water rinsed, and excess water was blotted using an

absorbent paper. This was followed by the application of an adhesive system (3M ESPE Adper Single bond 2, USA) and polymerized with light for 10 s. All cavities were restored with composite resin (Filtek Z550 A2 3M ESPE, USA) in increments of 2 mm and each layer was polymerized for 20 s with a LED (Valo, Cordless, Ultradent, Germany). Apices of teeth were covered by flowable composite (Competence Flow Willmann & Pein GmbH, Germany) to prevent the leakage of dye throughout the foramen. All external surfaces of crowns and roots were covered with two coats of nail varnish up to a distance of 1 mm beyond the margins of the restorations.

All specimens were subjected to thermocycling (SD Mechatronik Thermocycler, SD Mechatronik GMBH, Westerham, Germany) for 5,000 thermal cycles between 5 and 55 °C with a dwelling time of 30 s in each bath and a transferring time of 5 s. Following thermal cycling, teeth were immersed in 0.5% basic fuchsin dye solution for 24h. After this procedure, the teeth were washed and dried. Each tooth was sectioned bucco-lingually along the center of the restorations using with a slow-speed diamond saw (Isomet 1000, Buehler Ltd., Lake Bluff, IL, USA) machine. Microleakage of the restorations was measured under stereomicroscope (Leica M125, Leica, Heerbrugg, Switzerland) with a magnification of x40.

Dye penetrations at the occlusal and gingival margins were assessed by one examiner to determine the extent of microleakage according to a five-point scale as follows.¹⁵

0 = no leakage (figure 1)



Figure 1: Image of section with score 0 staining.

2 = Leakage up to 2/3 of gingival/occlusal walls (figure 3)

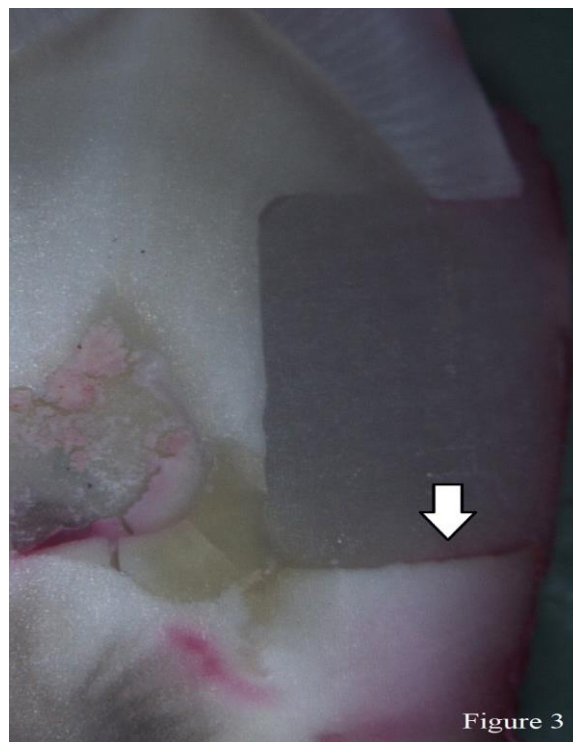


Figure 3: Image of section with score 2 staining

1 = Leakage up to 1/3 of gingival/occlusal walls (figure 2)

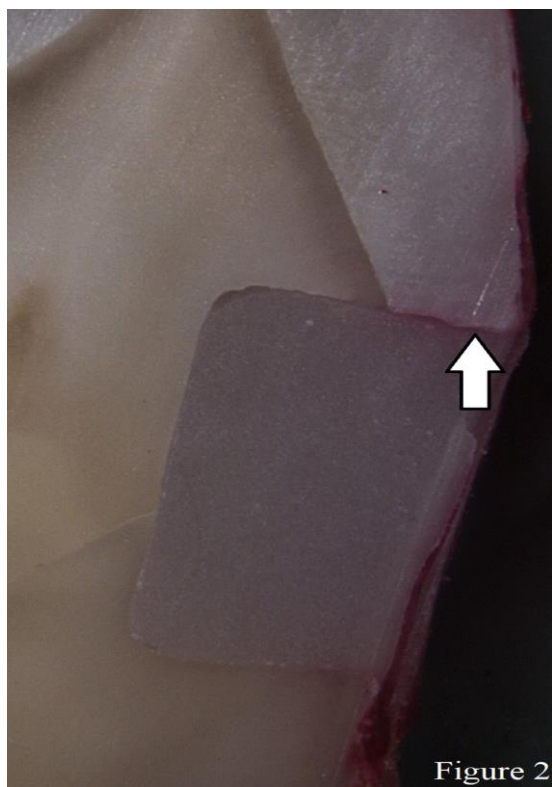


Figure 2: Image of section with score 1 staining

3 = Leakage up to base of the cavity (figure 4)

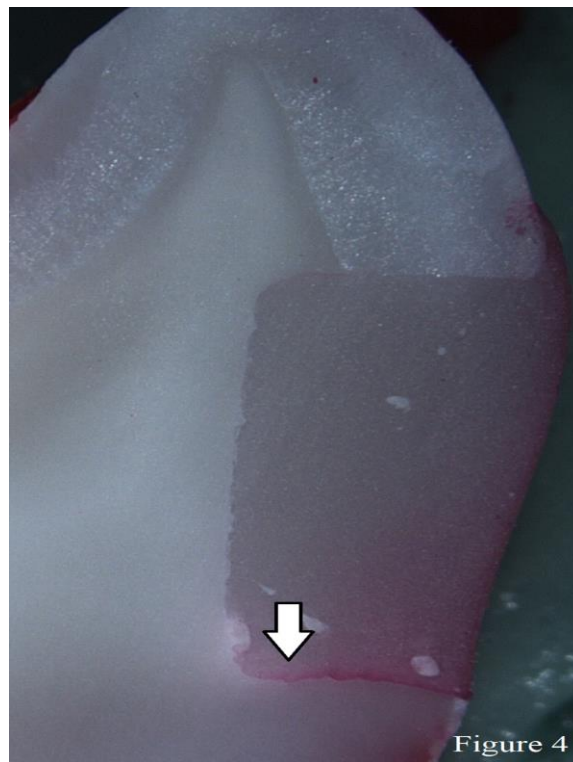


Figure 4: Image of section with score 3 staining

4 = Extensive dye penetration into axial wall (figure 5)

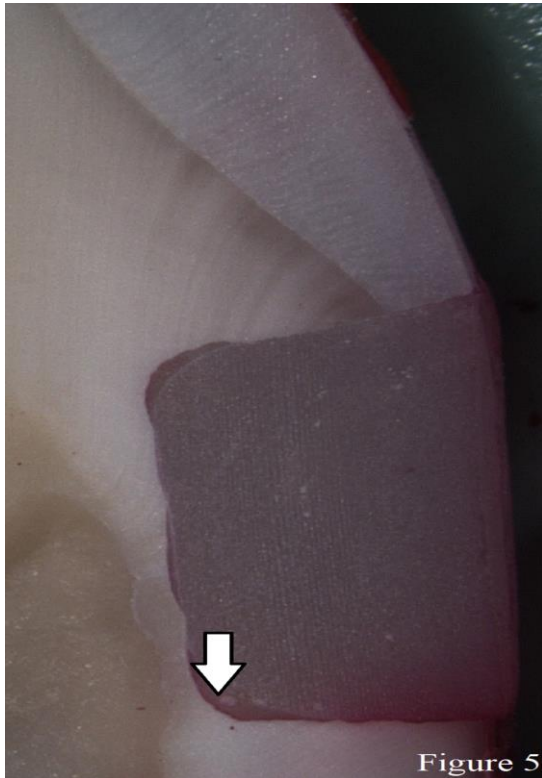


Figure 5: Image of section with score 4 staining

Statistical Analysis

Data was shown as median, interquartile range or frequency and percent. Prior to statistical analysis, the normality of the data was analyzed with Shaphiro Wilk test. Due to non-normal distribution of the data, statistical analysis was performed utilizing the Kruskal Wallis, One-way ANOVA followed by a Mann Whitney U test. Kruskal Wallis or Mann Whitney U test were used to compare the variables between/among groups. A p-value <0.05 was considered as significant. Analyses were performed using SPSS 19 (IBM SPSS Statistics 19, SPSS inc., an IBM Co., and Somers, NY).

RESULTS

The distribution of microleakage scores for groups and subgroups were represented on tables 1 and 2. At both on occlusal and gingival margins, microleakage could not be totally eliminated for all the groups.

Table 1. Distribution of microleakage scores according to the type of bleaching material and the inclusion of ozone on the occlusal and gingival margins of restorations.

Main Groups	Subgroups	Margin of Restoration	Scores					Median [25 - 75]
			0	1	2	3	4	
Opalescence Boost (40 % H ₂ O ₂)	Ozone	Occlusal	7	2	1	0	0	0[0-1] ^a
		Gingival	1	6	2	0	1	1[1-1] ^b
	Ozone-free	Occlusal	9	1	0	0	0	0[0-0] ^a
		Gingival	0	1	5	4	0	2[2-3] ^b
Whitess HP Blue (35 % H ₂ O ₂)	Ozone	Occlusal	8	2	0	0	0	0[0-0] ^a
		Gingival	1	6	1	1	1	1[1-1] ^b
	Ozone-free	Occlusal	7	1	1	1	0	0[0-1] ^a
		Gingival	1	2	3	3	1	2[1-3] ^b

Different superscript letters indicate significant difference among the groups.

Table 2. Distribution of microleakage scores according to the inclusion of ozone on the occlusal and gingival margins of restorations.

Subgroups	Margin of Restoration	Scores					Median [25-75]
		0	1	2	3	4	
Ozone	Occlusal	15	4	1	0	0	0[0-0.5] ^a
	Gingival	4	12	3	1	1	1[1-1] ^b
	Total	18	16	4	1	1	1[0-1] ^x
Ozone-free	Occlusal	16	2	1	1	0	0[0-0] ^a
	Gingival	1	3	8	7	1	2[1.5-3] ^b
	Total	18	5	9	8	1	1[0-2] ^x
Total	Occlusal	31	6	2	1	0	0[0-0] ^a
	Gingival	5	15	11	8	1	1[1-2] ^b

Different superscript letters indicate significant difference among the groups.

Statistical analysis (Kruskal Wallis and Mann Whitney U test) indicated that there were no significant differences between the two bleaching agents in terms of microleakage (p=0.675). Although there were no significant differences, the microleakage values of ozone-treated group were lower than values of ozone-free groups. In other words, the ozone treatment didn't significantly decrease the microleakage values (p=0.142). There were significant differences between the scores of microleakage at the occlusal and gingival margins regardless of the type of agent and ozone pre-treatment (p<0.001). Occlusal margins represented significantly lower

microleakage compared to gingival margins ($p < 0.001$).

DISCUSSION

With the increase of people's aesthetic expectations, the popularity of bleaching has been increasing in recent years. Vital office bleaching agents can be used successfully for discolored vital teeth.¹⁶ But, the effects of bleaching agents on tooth structure should be well known. Because, tooth whitening agents are able to penetrate into the tooth structure through the unsealed dentin margin at the tooth-restoration interface and thus, are capable of causing complications like tooth hypersensitivity and microleakage.¹⁷ This study investigated the effect of ozone application after office bleaching treatment on microleakage of composite resin restorations. The results of the present study stated that microleakage values of composite restorations following the use of different bleaching agents with or without ozone application were not statistically different. Neither the type of bleaching agent nor surface pre-treatment with ozone influenced microleakage values.

Bleaching agents can lead to the separation of polymer chains and break the double bonds because of these are highly unstable materials producing free radicals.¹⁸ The changes of the dental structure by oxidation of organic or inorganic elements is one of the probable side-effects of bleaching agents.¹⁹ Oxygen and oxidants can influence bonding to dental materials. Interactions between residual (per) oxide-related substances and restorative materials interfere with adhesion.²⁰ There have been reports regarding the interaction between bleaching agents and the bond strength of composite materials to enamel have shown that there is a significant decrease in the bond strength or increase in the microleakage when the composite resin is bonded to bleached enamel compared with unbleached enamel.²¹ The aim of laser bleaching treatment is to apply the most

effective energy source as prevention any adverse effect for achieving the latest power bleaching.²² It has been reported that bleaching treatment with laser irradiation improves both the whitening effect and preserve the change of enamel structure compared with bleaching treatment without laser-assisted.²³ Therefore, we aimed to evaluate the effects of laser-supported bleaching in the current study.

Ozone has the ability that lead to remineralization by gaining Ca and P ions through the tooth and remove proteins from carious lesions.²⁴ Dehydration of enamel is a reversible condition caused by ozone.²⁵ For this reason, removal of such residual moisture may notably improve adhesion of composite to the enamel structure. It has been reported that the dehydration resulting from ozone have no effect on the microhardness and surface-free energy of tooth surface, and have not influence on adhesion.²⁵ Cehreli *et al.*²⁶ reported that due to its strong oxidizing effect, ozone might have negative consequences on resin-tooth adhesion, since oxygen is a well-known polymerization inhibitor. Although the antibacterial efficacy of ozone treatment have been investigated in several studies^{27, 28}, there are only a few studies that have specifically evaluated the effects of ozone treatment on the microleakage and bond strength of adhesive systems.^{25, 29} Cehreli *et al.*³⁰ reported that fissure pretreatment with ozone led to a significant decrease in the microleakage values of both the bonded and conventional sealants, compared with their control (ozone-untreated) groups. In the current study, although there were no significant differences, the microleakage values of ozone-treated group were lower than values of ozone-less group. This effect may be due to the relatively high concentration of ozone in the present study.

The complex morphology of class V cavities with margins are partly in enamel as well as in root dentin presents difficulties for the restorative material.³¹ Mahrous *et al.*³² compared

the effect of the location of the gingival margin (enamel, dentin, or cementum) on the microleakage of posterior composite restoration and reported that the least microleakage was observed at the gingival margin located in the enamel while the most microleakage was detected at gingival margins located at cementum. Their results are partially in accordance with our result because our results demonstrated that occlusal (coronal) borders of the restorations represented lower microleakage compared to gingival margins. We assume that this is related with the higher bonding capacity of enamel to adhesive systems compared to dentine and cementum. Manhart *et al.*³³ also reported that marginal quality and sealing ability of adhesive systems to dentin was lower compared with occlusal margins.³³ According to the results of the current study, statistically significant differences were observed in dye penetration when occlusal margin and gingival margin were compared. All gingival margins showed higher microleakage score results compared to occlusal margins. These variations may also be explained by enamel thickness and structural differences. Increased tubule frequency and diameter at gingival margins may have interfered with adhesion of the restorations to dental hard tissues. Furthermore our findings are in accordance with the studies of Bektas *et al.*³⁴ and Moosavi *et al.*³⁵ who evaluated the effect of bleaching on the microleakage of Class V composite resin restorations. They reported that there was more microleakage in dentinal margins of composite restorations than in the occlusal margins in the test groups.

Microleakage should be evaluated to determine the clinical success of restorative materials and it is related to several factors, such as dimensional changes of materials due to polymerization shrinkage, thermal contraction, water absorption, mechanical stress and dimensional changes in tooth structure.³⁶ Different methods have been employed to evaluate microleakage around restorations in vitro such as including dye leakage method, the

use of color producing microorganisms, radioactive isotopes, the air pressure method, neutron activation analysis, electrochemical studies, scanning electron microscopy, thermal and mechanical cycling, and chemical tracers.³⁷ Thermocycling is an important procedure for testing the sealing ability of restorative material. Thermally induced stresses, which may lead to gap formation and microleakage, result from the mismatch between the coefficients of thermal expansion for the restorative material and the natural tooth structure.³⁸ Based on the studies, we used dye penetration method to investigate effects of bleaching treatment on microleakage after 5,000 thermal cycles.

CONCLUSION

Within the limitations of this study, the following conclusions were obtained:

- Applying immediately ozone after laser assisted vital bleaching didn't lead to decrease on microleakage of class V cavities. No significant differences were shown between the microleakage values of ozone-treated groups and ozone-free groups.
- At both occlusal and gingival margins, marginal microleakage was not eliminated completely in all the groups.

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Conflict of Interest

None

REFERENCES

1. Sari T, Celik G, Usumez A. Temperature rise in pulp and gel during laser-activated bleaching: in vitro. *Lasers Med Sci.* 2015;30(2):577-82.

2. Gurgan S, Cakir FY, Yazici E. Different light-activated in-office bleaching systems: a clinical evaluation. *Lasers Med Sci.* 2010;25(6):817-22.
3. Minoux M, Serfaty R. Vital tooth bleaching: Biologic adverse effects—A review. *Quintessence Int.* 2008;39(8).
4. Joiner A. The bleaching of teeth: a review of the literature. *J Dent.* 2006;34(7):412-9.
5. Anagnostou M, Chelioti G, Chioti S, Kakaboura A. Effect of tooth-bleaching methods on gloss and color of resin composites. *J Dent.* 2010;38 Suppl 2:e129-36.
6. Attin T, Hannig C, Wiegand A, Attin R. Effect of bleaching on restorative materials and restorations—a systematic review. *Dent Mater.* 2004;20(9):852-61.
7. Da Silva Machado J, Cândido MSM, Sundfeld RH, De Alexandre RS, Cardoso JD. The influence of time interval between bleaching and enamel bonding. *J Dent Res.* 2007;19(2):111-8.
8. Muller P, Guggenheim B, Schmidlin PR. Efficacy of gasiform ozone and photodynamic therapy on a multispecies oral biofilm in vitro. *Eur J Oral Sci.* 2007;115(1):77-80.
9. Schmidlin PR, Zimmermann J, Bindl A. Effect of ozone on enamel and dentin bond strength. *J Adhes Dent.* 2005;7(1):29-32.
10. Waldman G, Vaidyanathan T, Vaidyanathan J. Microleakage and resin-to-dentin interface morphology of pre-etching versus self-etching adhesive systems. *Open Dent.* 2008;2:120.
11. Krejci I, Lutz F. Marginal adaptation of Class V restorations using different restorative techniques. *J Dent.* 1991;19(1):24-32.
12. Türkün M, Türkün L. Effect of nonvital bleaching with 10% carbamide peroxide on sealing ability of resin composite restorations. *Int Endod J.* 2004;37(1):52-60.
13. Roubickova A, Dudek M, Comba L, Housova D, Bradna P. Effect of postoperative peroxide bleaching on the marginal seal of composite restorations bonded with self-etch adhesives. *Oper Dent.* 2013;38(6):644-54.
14. White DJ, Duschner H, Pioch T. Effect of bleaching treatments on microleakage of Class I restorations. *J Clin Dent* 2008;19(1):33-6.
15. Davidson C, Abdalla A. Effect of occlusal load cycling on the marginal integrity of adhesive Class V restorations. *Am J Dent.* 1994;7(2):111-4.
16. Haywood VB, Berry TG *Fundamentals of Operative Dentistry* 3ed. Schwarts RS, Summitt JB, Robbins JW editor. Chicago Quintessence; 2006. p. 437.
17. Khoroushi M, Fardashtaki S. Effect of light-activated bleaching on the microleakage of Class V tooth-colored restorations. *Oper dent.* 2009;34(5):565-70.
18. Malkondu O, Yurdagüven H, Say EC, Kazazoglu E, Soyman M. Effect of bleaching on microhardness of esthetic restorative materials. *Oper dent.* 2011;36(2):177-86.
19. McEvoy S. Chemical agents for removing intrinsic stains from vital teeth. II. Current techniques and their clinical application. *Quintessence Int.* 1989;20:379-84.
20. Torneck C, Titley K, Smith D, Adibfar A. Adhesion of light-cured composite resin to bleached and unbleached bovine dentin. *Dent Traumatol.* 1990;6(3):97-103.
21. Turkün M, Kaya AD. Effect of 10% sodium ascorbate on the shear bond strength of composite resin to bleached bovine enamel. *J Oral Rehabil.* 2004;31(12):1184-91.
22. Dostalova T, Jelinkova H, Housova D, Sulc J, Nemeč M, Miyagi M, et al. Diode laser-activated bleaching. *Braz Dent J.* 2004;15 Spec No:SI3-8.
23. Son JH, An JH, Kim BK, Hwang IN, Park YJ, Song HJ. Effect of laser irradiation on

crystalline structure of enamel surface during whitening treatment with hydrogen peroxide. *J dent.* 2012;40(11):941-8.

24.Baysan A, Lynch E. The use of ozone in dentistry and medicine. Part 2. Ozone and root caries. *Prim Dent Care.* 2006;13(1):37-41.

25.Celiberti P, Pazera P, Lussi A. The impact of ozone treatment on enamel physical properties. *Am J Dent.* 2006;19(1):67-72.

26.Cehreli SB, Guzey A, Arhun N, Cetinsahin A, Unver B. The effects of prophylactic ozone pretreatment of enamel on shear bond strength of orthodontic brackets bonded with total or self-etch adhesive systems. *Eur J Dent.* 2010;4(4):367-73.

27.Baysan A, Whiley RA, Lynch E. Antimicrobial effect of a novel ozone-generating device on micro-organisms associated with primary root carious lesions in vitro. *Caries Res.* 2000;34(6):498-501.

28.Baysan A, Lynch E. Effect of ozone on the oral microbiota and clinical severity of primary root caries. *Am J Dent.* 2004;17(1):56-60.

29.Dukić W, Lulić Dukić O, Milardović S. The influence of Healozone on microleakage and fissure penetration of different sealing materials. *Coll Antropol.* 2009;33(1):157-62.

30.Cehreli SB, Yalcinkaya Z, Guven-Polat G, Çehreli ZC. Effect of ozone pretreatment on the microleakage of pit and fissure sealants. *J Clin Pediatr Dent.* 2010;35(2):187-90.

31.Dietrich T, Lösche A, Lösche G, Roulet JF. Marginal adaptation of direct composite and sandwich restorations in Class II cavities with cervical margins in dentin. *J Dent.* 1999;27(2):119-28.

32.Mahrous AI, Eltiti HA, Ahmed IM, Alagha EI. Effect of different gingival margin restorations of class II cavities on microleakage: an in-vitro study. *Electronic physician.* 2015;7(7):1435.

33.Manhart J, Chen HY, Mehl A, Weber K, Hickel R. Marginal quality and microleakage of adhesive class V restorations. *J dent.* 2001;29(2):123-30.

34.Bektas OO, Eren D, Akin GG, Sag BU, Ozcan M. Microleakage effect on class V composite restorations with two adhesive systems using different bleaching methods. *Acta Odontol Scand.* 2013;71(3-4):1000-7.

35.Moosavi H, Moghaddas MJ, Ghoddusi J, Rajabi O. Effects of two antioxidants on the microleakage of resin-based composite restorations after nonvital bleaching. *J Contemp Dent Pract.* 2010;11(6):E033-40.

36.Staninec M, Mochizuki A, Tanizaki K, Jukuda K, Tsuchitani Y. Interfacial space, marginal leakage, and enamel cracks around composite resins. *Oper dent.* 1986;11(1):14.

37.Taylor M, Lynch E. Microleakage. *J dent.* 1992;20(1):3-10.

38.Versluis A, Douglas WH, Sakaguchi RL. Thermal expansion coefficient of dental composites measured with strain gauges. *Dent Mater.* 1996;12(5):290-4.

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