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Comparative Evaluation of Cavities Disinfected with Ozone Gas and Photodynamic Therapy and Restored with Two Different Reinforced Glass Ionomer Cements on Microleakage and Color Change

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ABSTRACT

The aim of this study is to examine the effect of chlorhexidine (CHX), ozone and photodynamic (PDT) therapy on the microleakage of reinforced glass ionomer cement and on tooth discoloration. The class V cavities were opened on the buccal faces of 96 extracted molar teeth and the samples were divided into four groups (n = 24). Before and after cavity disinfection, color measurement of teeth was determined by using a spectrophotometer. The groups were separated into two subgroups and restored with ACTIVA and Giomer. One thousand thermal cycles were applied to the teeth at 5–55 C. After the penetration of 0.5% basic fuchsin, teeth were examined with a stereomicroscope and SEM. The data were statistically analyzed using the Mann Whitney U-test and the Kruskal Wallis test. The differences were statistically significant among the cavity disinfectants on the occlusal edge in the ACTIVA groups ($p < .05$), There was no significant difference considering cavity disinfectants on occlusal and gingival margins in the Giomer groups ($p > .05$). While ozone gas showed an acceptable color change in teeth, CHX and PDT caused the discoloration in teeth. It was concluded that the Ozone gas does not affect the microleakage of the reinforced glass ionomer cements and does not cause detectable color change. CHX and PDT reduce the microleakage of ACTIVA at the enamel level.

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Ozone; dentistry; microleakage; glass ionomer cement; color change

Introduction

Cariogenic bacteria are responsible for the development of caries. Therefore, microorganisms responsible for caries must be removed from the cavity wall, smear layer and dentinal tubules before beginning restorative treatment. Bacteria remaining in the dentin tissue can cause pulpal inflammation, recurrent caries and postoperative sensitivity (Imazato et al. 2001). In order to remove all bacteria from the cavity wall and reduce the possibility of secondary caries, the use of antibacterial solutions for disinfection of cavities has been recommended (Dalkilic et al. 2012; Magni et al. 2008). Among the various chemicals tested as cavity disinfectants, there are chlorhexidine digluconate (CHX), sodium hypochlorite, hydrogen peroxide and iodine potassium iodide (Ercan et al. 2009; Sharma, Nainan, and Shivanna 2009). Alternatively, different treatment procedures such as lasers, ozone and photodynamic therapy have been evaluated for their antimicrobial effects (Baysan and Lynch 2006; Bergmans et al. 2006; Wilson 1994).

The solution of 2% CHX is one of the most widely used cavity disinfectant solutions in clinical dentistry as a broad-spectrum antibacterial agent (Miranda et al. 2014; Varoni et al. 2012). CHX have a bacteriostatic and bactericidal effect on the bacterial cell membrane (Kanisavaran 2008). In addition to its antibacterial effect, another important aspect of chlorhexidine use is that it preserves the dentin composite bond strength by acting as a matrix metalloproteinase (MMP) inhibitor responsible for the degradation of all extracellular matrix components (Aykut-Yetkiner et al. 2015).

Ozone is a disinfectant with strong antibacterial effect, damaging the cytoplasmic membrane and cell wall of bacteria, fungi, protozoa and viruses in gaseous or liquid phase (Bocci 2006). There are several studies evaluating the effects of ozone use on carious lesions and in reducing the number of microorganisms (Baysan, Whiley, and Lynch 2000; Brazzelli et al. 2006). Recently, it has been reported that ozone application was used in dental hard tissues as a cavity disinfectant before adhesive restorations (Azarpazhooh and

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Limeback 2008; Schmidlin, Zimmermann, and Bindl 2005).

Antimicrobial photodynamic therapy (PDT) is a new technique assumed to control dental caries successfully. The photo-activated disinfection is used in combination with the light of a wavelength and a photosensitizer fluid, which produces single oxygen and other radical species to remove microorganisms from dentin tubules, ensuring rapid and selective destruction of target cells (Raghavendra, Koregol, and Bhola 2009). Phenothiazine-based dyes such as Toluidine Blue, Methylene Blue, and Malachite Green are currently the most commonly used photosensitizers in PDT (Carvalho et al. 2011; Garcez et al. 2008; Nunes et al. 2011). However, because they are dyes, these photosensitizers may change the color of the tooth structure (Carvalho et al. 2011).

Glass ionomer cement (GIC) has advantages of ease of use, anticariogenic and remineralization potentials due to its fluorine ion release and rechargeable properties. However, it has disadvantages of low mechanical and physical properties (Croll and Nicholson 2002). Giomer, which has recently been developed to gain the advantages of GIC to resin materials, contains pre-reacted glass ionomer particles (PRG) (Ikemura et al. 2008). In addition, ACTIVA is a novel restorative material which is a combination of GIC and composite resin, containing bioactive glass particles (Croll, Berg, and Donly 2015).

There has been insufficient data in the literature regarding the microleakage of these restorative materials. Moreover, there is a lack of study on the effect of cavity disinfectants on the microleakage of these restorative materials.

Therefore, the aim of this study is to evaluate the effect of different cavity disinfectants on reinforced glass ionomer cements in terms of microleakage and discoloration. The hypothesis of this study is that the cavity disinfectants may not affect the microleakage of two different reinforced glass ionomer cements and may not cause any tooth discoloration.

Materials and methods

Microleakage test

Ninety-six third molars extracted due to orthodontic reasons or periodontal diseases used in this study. The teeth were kept in distilled water at room temperature. Standard Class V cavities of 2 mm on the buccal surfaces of the teeth, 2 mm in the mesiodistal and 3 mm in the occluso-gingival size were prepared. The gingival edge was shaped 1 mm above the enamel-cementum border. A sharp diamond fissure bur was used in a high-speed handpiece with air and water spray for cavity preparation. One bur was used for every five cavity preparations. The teeth were randomly divided into four groups. Each group was divided into two subgroups from randomly selected teeth (n = 12).

Group 1: 2% CHX (Imicryl, Konya, Turkey) was applied on the prepared dentin surface for 30 seconds with a disposable applicator and dentin surface was dried with cotton pellets.

Group 2: An ozone generator (Ozone DTA, Apoza, New Taipei, Taiwan) was used to generate ozone gas. The Ozone gas was applied to the cavity surfaces in contact mode for 90 seconds at the third power level with the probe tip number 3 which supplies 235 ppm gas concentration

Group 3: 0.1 mg/mL of toluidine blue was applied into the cavity and kept in the dark for 5 minutes. Diode laser was applied with a 0.8 W fiber optic tip in continuous mode which provides monochrome light at 810 nm wavelength. The tip was used without contact to the dentine surface for 30 seconds in helicoidal movements at a speed of approximately 2 mm/s. The dye was then aspirated from the specimens and the dentin surface was washed with distilled water and air dried for 5 seconds.

Group 4: Control group. No disinfectant application.

After the disinfectant applications, Universal bond (3 M ESPE, Neuss, Germany) was applied to the dentine surfaces for 10 seconds. It was dried with air for 10 seconds and irradiated with a 1200 mW/cm^2 power LED (Freelight Elipar II, 3 M, MN, USA) device for 10 seconds. Giomer (Shofu, Kyoto, Japan) was applied to the cavity using a hand tool and irradiated for 20 seconds. ACTIVA was applied to the cavity with a gun and irradiated for 20 seconds. When the restorations were completed, all groups were polished using medium, fine and superfine aluminum disks (Optidisc, Kerr, CA, USA), respectively.

All teeth were placed in the thermal cycle device and 1000 cycles were applied at 5–55°C (20 seconds application, 10 seconds waiting). After thermal cycle, the apical foramen was covered with flowable composite and the teeth were painted with two layers of nail polish, except the restoration and 1 mm surrounding of it. The teeth were kept in 0.5% basic fuchsin solution in a 37°C incubator for 24 hours and washed and embedded vertically in acrylic blocks They were cut in the buccolingual direction by the precision cutting device (Accutom 10, Struers, OH, USA). The sections were examined under a stereomicroscope at ×40 magnification (Figure 1). Microleakage scoring for both the gingival and occlusal walls was completed by consensus of two researchers (Table 1.) (Santini et al. 2004).

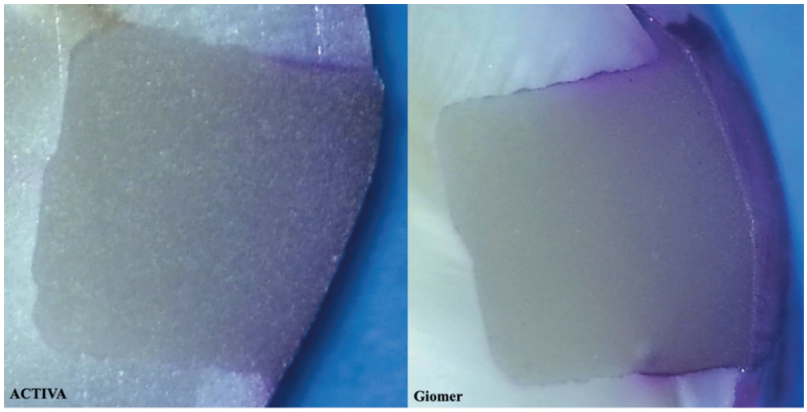


Figure 1. Stereomicroscope images of cross-sectional samples from ACTIVA and Giomer groups for scoring of microleakage based on dye penetration at occlusal and gingival margin.

Table 1. The microleakage score scale.

Score 0	No dye penetration
Score 1	Dye penetration up to one-third of the cavity wall
Score 2	Dye penetration up to two-third of the cavity wall
Score 3	Dye penetration without axial wall
Score 4	Dye penetration in the axial wall

A random sample was selected from each group to examine the surface morphology between the restorative material and dental hard tissue in the coronal section of the tooth using a scanning electron microscope (SEM) (FEI, Quanta FEG 250, Germany).

Color measurement

After the cavity was prepared, the first color measurement of the teeth was determined by using a spectrophotometer (VitaEasyshade, Vita Zahnfabrik, Bad Sackingen, Germany). After cavity disinfection, second color measurement was determined on the teeth at the same point. The calculation of the $CIEDE_{2000}$ color difference (ΔE_{00}) was done by using an Excel spreadsheet application of the $CIEDE_{2000}$ color difference formula provided by Sharma (G. Sharma, Wu, and Dalal 2005). The parametric factors of the formula were set to 1.

Kruskal–Wallis variance analysis test was used for the multiple comparison of microleakage. The Mann–Whitney U-test was used for paired comparisons between groups and within groups of microleakage. The normality assumption of color change data was analyzed by the Shapiro–Wilk test and then multiple comparisons with the Kruskal–Wallis test and paired comparisons with the Mann–Whitney U-test were determined.

Results

Microleakage

The microleakage score values of the materials on the occlusal and gingival walls are shown in Figures 2 and 3. The median, minimum, and maximum values for microleakage variables in each group are represented in Tables 2 and 3. The microleakage was seen in all groups. The statistical analysis showed that there was no significant differences among the cavity disinfectants for both of the restorative materials at the gingival edge. However, a significant difference was found between the cavity disinfectants on the occlusal wall of the ACTIVA group.

The occlusal microleakage score was evident between the ACTIVA groups ($p = .002$) when the Kruskal–Wallis non-parametric test was employed. CHX and PDT cavity disinfectants reduced microleakage of the ACTIVA group comparing control group ($p = .002$), ($p = .006$), respectively. There was no difference between ozone and control groups on the occlusal wall ($p > .05$). The Ozone applied group increased the microleakage in the occlusal wall comparing cavities disinfected with CHX and PDT; ($p = .007$), ($p = .029$), respectively.

No statistically significant differences for cavity disinfectants were found on both occlusal and gingival walls, when Giomer groups were compared with the Kruskal Wallis test ($p > .05$).

When the same disinfectants applied Giomer and ACTIVA groups were compared, the Giomer group showed less leakage than the ACTIVA group on the occlusal wall of the ozone applied groups ($p = .000$). In the control group, the Giomer showed less leakage in the occlusal wall ($p = .007$). There was no difference between the two restorative materials in terms of microleakage on the gingival wall.

Table 2. Median values of microleakage at occlusal wall in the groups.

Groups	n	Median	Min	Max
CHX ACTIVA	12	1	0	3
Ozone ACTIVA	12	2	1	4
PDT ACTIVA	12	1	0	3
Control ACTIVA	12	2	1	4
CHX Giomer	12	0.5	0	1
Ozone Giomer	12	0	0	1
PDT Giomer	12	0	0	3
Control Giomer	12	1	0	2

CHX: Chlorhexidine, PDT: Photodynamic therapy, Min: Minimum, Max: Maximum.

Table 3. Median values of microleakage at gingival wall in the groups.

Groups	n	Median	Min	Max
CHX ACTIVA	12	2	0	3
Ozone ACTIVA	12	2	0	4
PDT ACTIVA	12	1.5	0	4
Control ACTIVA	12	2	1	4
CHX Giomer	12	2.5	0	4
Ozone Giomer	12	2	0	3
PDT Giomer	12	2	0	3
Control Giomer	12	3	0	4

CHX: Chlorhexidine, PDT: Photodynamic therapy, Min: Minimum, Max: Maximum.

Figure 4 indicates the morphology of the surface between the reinforced glass ionomer cement and dental hard tissues for each group by using SEM.

Color change

Mean ΔE_{00} values and standard deviations of color changes are shown in Figure 5 and Table 4. According to the Kruskal–Wallis multiple comparison, there was a statistically significant difference among the groups (p = .000). As a result of binary comparisons was determined by Mann–Whitney U-test, the lowest color change was observed in the ozone group, and this difference was statistically significant compared to PDT and CHX groups (p = .000) (p = .005) respectively. The difference between the CHX and PDT groups was also found statistically significant (p = .009).

Discussion

The effects of disinfectant methods actively used on leakage of the current restorative materials were investigated in this study. Class V cavities were prepared to assess the microleakage of the enamel and dentin edge simultaneously. The cavity disinfectants had no effect on the microleakage of the Giomer group at the occlusal and gingival edges. However, by using CHX and PDT, it was observed that the microleakage was reduced at the occlusal edge in the ACTIVA group. The results of this study showed that this type of disinfectant affects microleakage. Discoloration occurred in the teeth during disinfection using dyes. Due to these results, the H_0 hypothesis tested was rejected.

ACTIVA is a bioactive-restorative material that provides long-term clinical benefits by reacting to the changes in the mouth through ion exchange (Amaireh, Al-Jundi, and Alshraideh 2019). In a microleakage study, higher microleakage scores were observed in ACTIVA than composite resin without a statistically important difference (Amaireh, Al-Jundi, and Alshraideh 2019). In another study, the microleakage on the gingival edge of ACTIVA was increased compared to the occlusal edge (Ebaya, Ali, and Mahmoud 2019). These results are in accordance with the results of our study, also the microleakage of the ACTIVA group

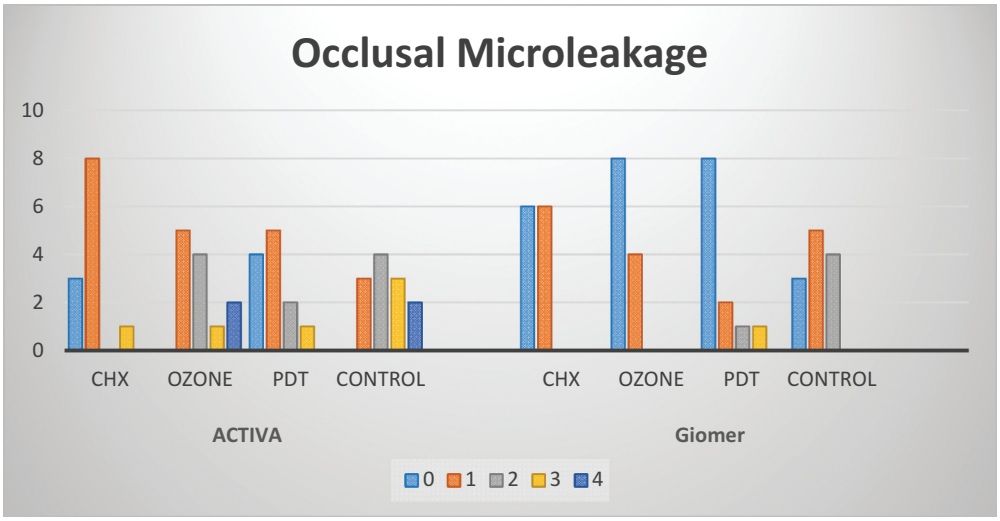


Figure 2. Microleakage score of occlusal wall.

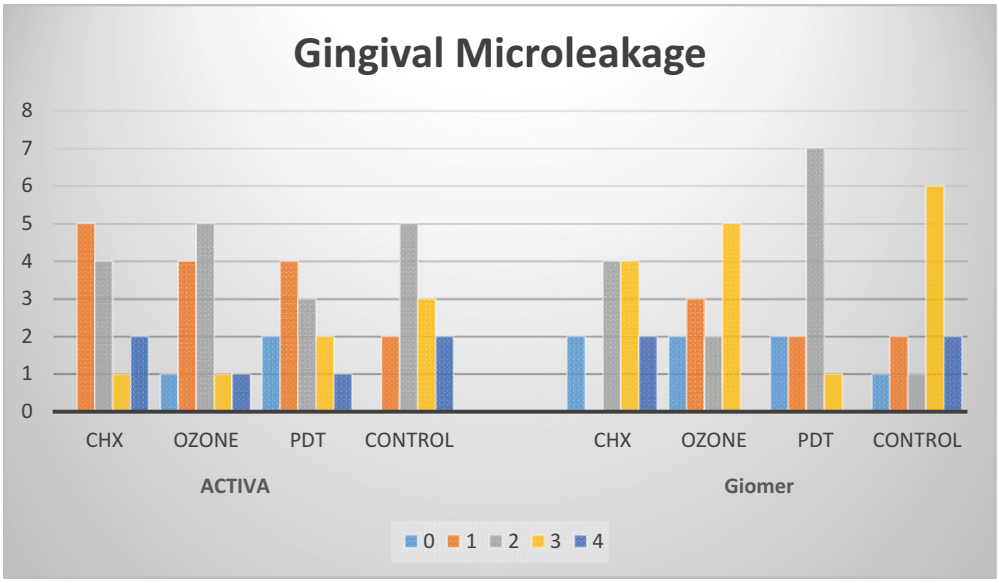


Figure 3. Microleakage score of gingival wall.

at the occlusal edge was found not to be as successful as the Giomer group.

Giomers which are a new class of materials are aimed to combine the best aspects of composite resins and GICs. Giomer showed the most leakage in various studies comparing different GIC types and composite resins (Rusnac et al. 2019; Walia et al. 2016). In our study, microleakage scores at both occlusal and gingival edges of the Giomer group were found to be lower than those of the ACTIVA group. However, when all the Giomer scores were considered, they were quite high in terms of microleakage. This may be due to reduced marginal adaptation of the Giomer by polymerization shrinkage or the use of a self-etch adhesive system. In a study, it is thought that the main cause of marginal deterioration of Giomer restorations is hygroscopic expansion, which is a unique feature of this restorative material (Sunico, Shinkai, and Katoh 2005).

Evaluating the Giomer and cavity disinfectants, the group applied 2% CHX showed the most leakage. The authors attributed this to the adverse effect of chlorhexidine on the binding of self-etch bonds (Kimyai et al. 2017). A study was carried out by using 2% CHX and two different cavity disinfectants. It was concluded that disinfectants did not affect the leakage of the Giomer (Mutluay and Mutluay 2019). We observed that cavities restored with Giomer had similar microleakage scores with or without different cavity disinfection methods. These results were found to be consistent with the previous studies. However, there is a lack of study on the use of ACTIVA together with the cavity disinfectants in the literature. In our study, CHX application enabled the ACTIVA group to show less leakage at the occlusal edge.

Ozone is a natural disinfectant agent which has a strong antibacterial, antiviral and antifungal effect. The application of ozone water for 10 seconds was reduced the most of the bacteria together with *S. mutans* and *S. sobrinus* and the 20 seconds of application destroyed 99.9% of the microorganisms on the carious dentin surface (Baysan, Lynch, and Grootveld 2001). The studies of the effect of ozone and 2% CHX with different resin materials on the microleakage found that there was no difference in leakage between the occlusal and gingival edges of the cavity disinfectants (Cellik and Bahsi 2019; Güneş et al. 2014). The effects of sodium hypochlorite, CHX, ozone gas and boric acid at different concentrations (1%, 3%, 5% and 7%) as cavity disinfectants were compared on the microleakage of composite restorations. Ozone application showed lower scores than CHX and higher scores than boric acid at the occlusal and gingival edges (Cangul et al. 2019). In other studies, no effect of ozone application on the microleakage was found (Celiberti, Pazera, and Lussi 2006; Dukić, Lulić Dukić, and Milardović 2009). Also, there was no study on the effect of ozone application on microleakage of reinforced glass ionomer cements.

This study is one of the first studies to investigate the effect of ozone on the microleakage of Giomer and ACTIVA, which were the current reinforced glass ionomer-containing materials. In our study, it was observed that ozone application had no effect on microleakage at the occlusal and gingival edges of the Giomer and ACTIVA. Free oxygen radicals released by ozone application may not have affected the microleakage of the materials since they are not high enough to affect the

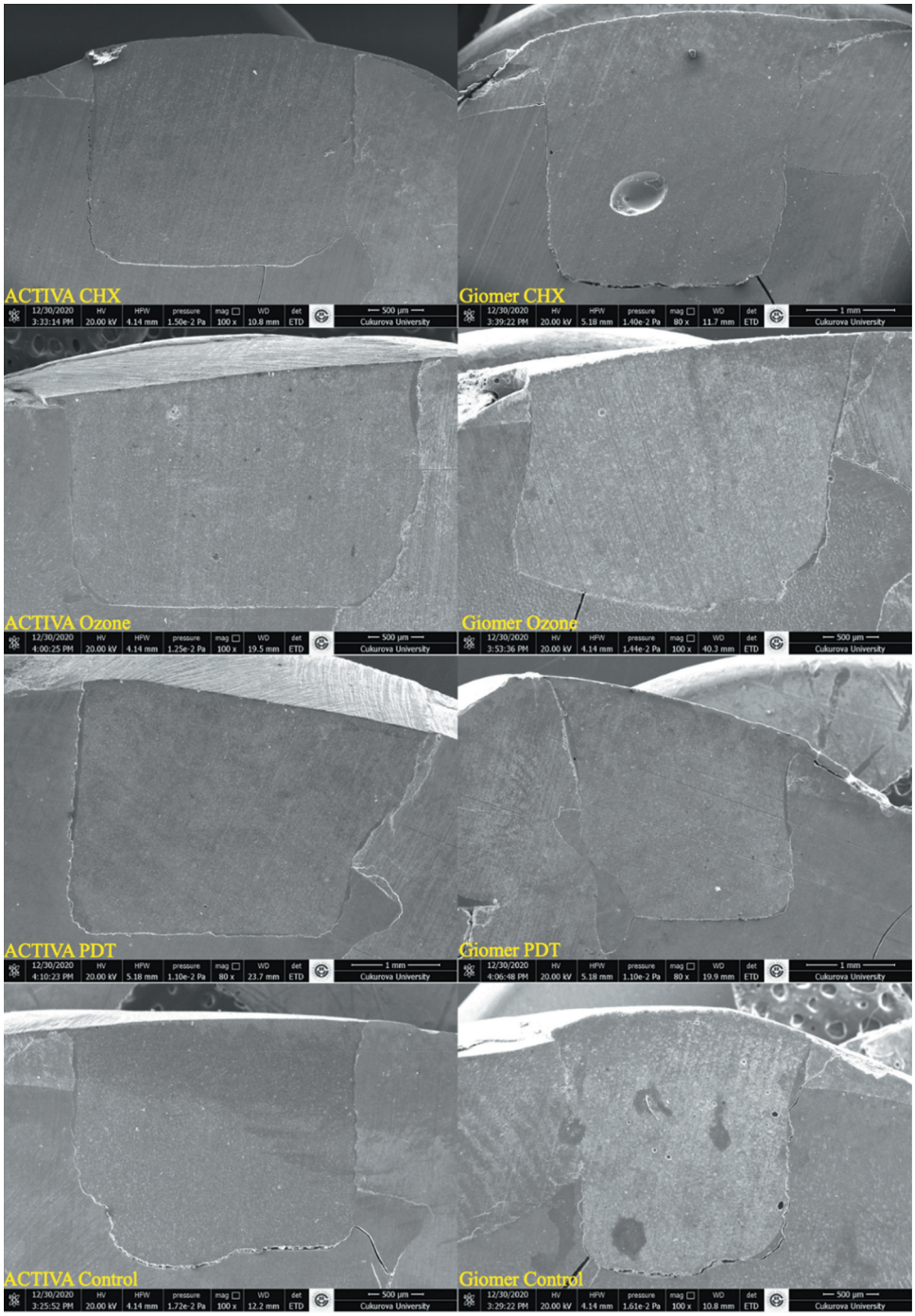


Figure 4. SEM images display a representative coronal section view of class V restoration of Activa and Giomer groups with cavity disinfectants which are CHX, ozone, PDT and control.

binding. However, the occlusal edge of the Giomer showed less leakage than the ACTIVA compared to the ozone application groups because giomer contains more resin materials in terms of the mechanical properties.

Wilson et al.(Wilson 1994) reported the use of PDT as a new method to eliminate cariogenic bacteria and plaque-forming organisms including a photosensitizer and a low-power laser beam application. As a result of a disinfection study, using a light-emitting diode and toluidine blue in biofilms showed a 95% of decrease in *Streptococcus mutans*, *S. sanguis* and *S. sorbinus* (Zanin et al. 2005). Comparing the effect of composite resin on microleakage of Nd-YAG laser, PDT and diode laser, PDT had a lower microleakage score than other groups

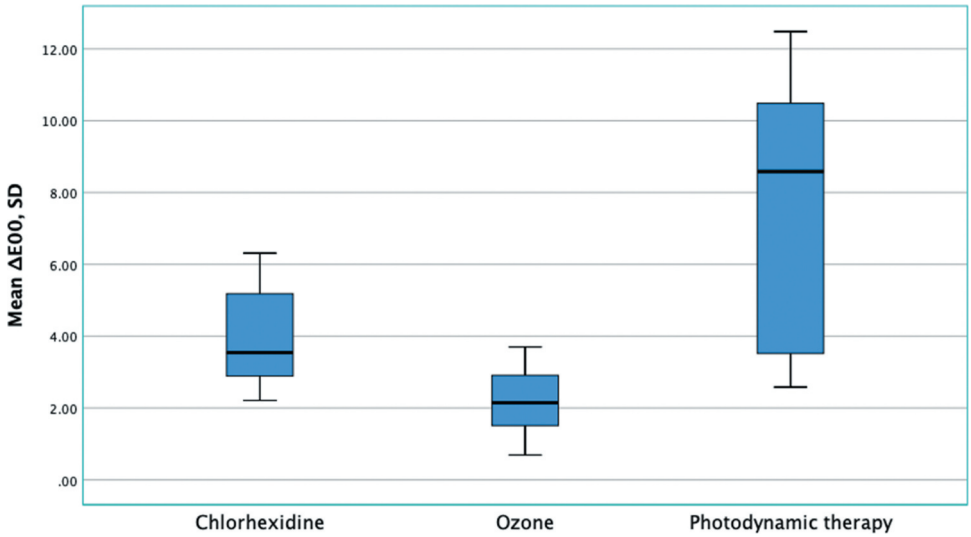


Figure 5. Box plot data of ΔE_{00} values.

Table 4. Mean and standard deviation of the values of ΔE_{00} in the groups.

Groups	Mean	SD	Min	Max
CHX	3.9905[a]	1.2895	2.21	6.31
OZONE	2.1823[b]	0.8411	0.69	3.70
PDT	7.5109[c]	3.6662	2.58	12.48

Letters [a, b, c] indicate the statistically differences between groups. CHX: Chlorhexidine, PDT: Photodynamic therapy SD: Standard deviation, Min: Minimum, Max: Maximum.

without a significant difference (Oskoee et al. 2013). Usage of a 660 nm wavelength diode laser and methylene blue combination had no effect on the microleakage in etch-and-rinse and self-etch systems (Madani et al. 2018). They stated that this result may be due to the fact that free oxygen radicals released were not high enough to affect adhesion. Furthermore, PDT works with low power values, which have minimum thermal effects on the dentin compared to other lasers. All these positive reasons caused PDT as one of the main disinfection methods. In this study, PDT did not have any effect on the microleakage of the Giomer while decreasing microleakage of the ACTIVA at the occlusal edge.

This may be due to the difference in the bonding strength of the enamel and dentine materials. The gingival edge of class V cavities is mainly located in dentin which has higher moisture. This may affect dentin bonding and increase microleakage at the gingival margin. Studies on microleakage of reinforced glass ionomer cements after PDT are limited. Since this method is new, more studies on PDT are needed.

Studies on PDT have focused on reducing bacterial load so far. However, photosensitizers may cause staining of teeth. In this study, $CIEDE_{2000}$, one of the CIE-L*A*B*-System, was used as a method of monitoring color changes. $CIEDE_{2000}$ was reported to be a formula with color differences and perceptibility better adapted to human visual values (Acar et al. 2016; Barutçugil et al. 2019). $CIEDE_{2000}$ (DE_{00}) and color differences were evaluated in terms of perceptibility and clinical acceptability thresholds. In this study, detectability threshold was accepted as DE_{00} = 1.28 units and clinical acceptability threshold as DE_{00} = 2.24 units (Ghinea et al. 2010).

In the second part of our study, where the dental coloration of ozone, CHX and PDT were examined, the color change caused by PDT, CHX and ozone was found to be E_{00} = 7.51, 3.99, 2.18, respectively. Clinically unacceptable coloration was observed in PDT and CHX groups. There was a lack of study examining the effect of the application of PDT, ozone and chlorhexidine as cavity disinfectants on dental discoloration. However, it was reported that the use of CHX mouthwashes may cause discoloration on the teeth (Slot et al. 2014). In studies examining the effect of methylene and toluidine blue on discoloration in PDT, it was stated that photosensitizers cause tooth discoloration (Costa et al. 2016; Figueiredo et al. 2014).

In this study, a thermal cycle was used to create microleakage at the tooth-restoration interface. However, there are limitations in the inability to imitate

biological changes such as chewing forces, chemical attacks caused by acid and enzymes that impair the durability of the restoration in the oral cavity. Moreover, reinforced glass ionomers did not carry out ion exchange with minerals in saliva is another limitation. Further research may confirm these results by conducting in vivo studies.

Conclusion

Within the limitations of this study, Giomer was found to be more successful in microleakage than ACTIVA. CHX and PDT contribute positively to ACTIVA and enamel bonding. CHX and PDT applications cause a clinically unacceptable color change in a tooth. It was concluded that the cavity disinfection method with ozone can be used without affecting the material adhesion and tooth color.

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Declaration of interest statement

The authors declare no conflict of interest regarding any of the materials used in this study.

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