European Journal of Research in Dentistry 2023; 7(3): 115-121
DOI: http://dx.doi.org/10.29228/erd.55

ORIGINAL ARTICLE / ARAŞTIRMA MAKALESİ

The Effect of Cavity Disinfectant on Microleakage of Self-adhesive Composite Restorations in Class V Cavities

Sınıf V Kavitelerde Kavite Dezenfektanlarının Self Adeziv Kompozit Restorasyonların Mikrosızıntısı Üzerine Etkisi

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ABSTRACT

Objectives: This *in vitro* study was aimed to evaluate the effect of chlorhexidine digluconate (CHX) containing cavity disinfectant on microleakage of Class V self-adhesive resin-based composite restorations.

Materials and Methods: Forty non-beveled Class V cavities (4 mm height x 2 mm width x 2mm depth) 1 mm above the cemento-enamel junction were prepared on lingual and buccal surfaces of 20 molar teeth. Samples were randomly divided into 4 groups (n=10); and lingual cavities were disinfected with 2% CHX (Bisco). Cavities were restored using 2 different self-adhesive composites: Vertise Flow (Kerr); Activa BioACTIVE (Pulpdent) according to manufacturer's instructions. Following 20 s polymerization with Valo (Ultradent) LED curing light, finishing and polishing were performed using Finishing Discs (Bisco). Teeth were coated with a nail polish excluding the restoration area and aged in distilled water at 24°C for 6 months. After immersion of the samples in 2% methylene blue solution, they were sectioned longitudinally in buccolingual direction with a diamond saw (Isomet 1000, Buehler). Microleakage on occlusal/gingival margins were scored under x8 and x20 magnification using a stereomicroscope (Leica MZ7.5). For statistical evaluation**,** Chi-square test was used. The significance level was set at $p < 0.05$.

Results: No statistically significant difference was detected between the total microleakage scores at occlusal and gingival margins (p=0.735; 0.944). Likewise, there was no significant difference between the gingival/occlusal margins of the restorations (p=0.216). CHX application did not show any statistically significance between microleakage values in Class V cavities restored with Vertise Flow or Activa BioACTIVE. (p=0.942; 0.577).

Conclusions: CHX cavity disinfectant did not prevent microleakage in Class V cavities restored with self-adhesive composites.

Keywords: Bioactive material, Cavity disinfectant, Class V cavity, Microleakage, Self-adhesive composite

ÖZ

Amaç: Bu *in vitro* çalışmanın amacı, klorheksidin diglukonat (CHX) içeren kavite dezenfektanının, sınıf V self-adeziv rezin bazlı kompozit restorasyonların mikrosızıntısı üzerindeki etkisini değerlendirmektir.

Gereç ve Yöntemler: 20 insan azı dişinin hem bukkal hem de lingual yüzeylerine mine-sement birleşiminin 1 mm üzerinde olan 40 adet standart sınıf V kavite (4 mm yükseklik x 2mm genişlik x 2mm derinlik) hazırlandı. Örnekler rastgele 4 gruba ayrıldı (n-10); tüm lingual kaviteler %2 CHX (Bisco) ile dezenfekte edildi. Kaviteler 2 farklı self-adeziv kompozit (Vertise Flow, Kerr; Activa BioACTIVE, Pulpdent) kullanılarak üretici talimatlarına göre restore edildi. Valo (Ultradent) LED ışıklı cihaz ile 20 sn polimerizasyon sonrası Finishing Discs (Bisco) kullanılarak bitim ve polisaj işlemleri yapıldı. Dişlerin restorasyon alanı dışında kalan yüzeyleri şeffaf oje ile kaplandı ve distile suda oda sıcaklığında (24°C) 6 ay yaşlandırıldı. Numuneler %2'lik metilen mavisi solüsyonuna bir saat daldırıldıktan sonra, hassas kesme cihazı (Isomet 1000, (Buehler)) ile bukkolingual yönde boylamasına kesitler alındı. Oklüzal/gingival mikrosızıntı stereomikroskop (Leica MZ7.5) kullanılarak x8 ve x20 büyütme altında skorlandı. İstatistiksel değerlendirme için, ki-kare testi kullanıldı ve anlamlılık düzeyi $p<0.05$ olarak belirlendi.

Bulgular: Kaviteye CHX uygulamasına göre restorasyonların oklüzal ve gingival mikrosızıntı değerlerinde istatistiksel olarak anlamlı fark bulunmadı (p=0.735; 0.944). Benzer şekilde, gingival ve oklüzal kenarlardaki mikrosızıntı seviyeleri arasında anlamlı fark saptanmadı (p=0.216). Vertise Flow veya Activa BioACTIVE

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Submitted / Gönderilme: 08.03.2023 **Accepted/Kabul:** 25.09.2023

ile restore edilen CHX ile muamele edilmiş ve edilmemiş sınıf V kavitelerde mikrosızıntı değerleri arasında istatistiksel açıdan anlamlı fark tespit edilmedi (p=0.942; 0.577).

Sonuç: CHX kavite dezenfektanı, self-adeziv kompozitlerle restore edilen sınıf V kavitelerde mikrosızıntıya engel olmamıştır.

Anahtar Kelimeler: Biyoaktif materyal, Kavite dezenfektanı, Mikrosızıntı, Self adeziv kompozit, Sınıf V Kavite

INTRODUCTION

Restorative materials in dentistry, re-establish functional, esthetic and biological properties of the teeth (Mishra et al., 2018). Resin-based composites are generally used for cervical lesions as these materials bond to the tooth structures and composite restorations are esthetically pleasing (Lokhande et al., 2014).

The longevity of composites depends on microleakage and resistance to masticatory forces (Mishra et al., 2018). Microleakage is one of the critical factors causing failure of resin-based restorations (Guo et al., 2016). Shrinkage can occur during the polymerization of resin-based restorative materials, if the adhesion force is not strong enough to resist the effects of the shrinkage, gap formation will occur between the tooth and the restoration. Microorganisms and oral fluids leak into the cavity from this gap (Nilgun Ozturk et al., 2004; Kleverlaan & Feilzer, 2005). Deeper invasion of microorganisms in the tooth structure may cause secondary caries, post operative sensitivity and inflammatory changes in the pulp (Silveira de Araújo et al., 2006). Marginal sealing depends on many factors such as: restoration technique, mechanical and physical properties of the material, etc (Van Ende et al., 2017). Recently, self-adhesive and flowable composites have been advanced as a new category. According to the manufacturer's instructions, these composites do not require prior etching or bonding. (Rahimian-Imam et al., 2015). They contain acidic monomers and manufacturer's claim these products provide marginal sealing and prevent overwetting, overdrying, and overetching (Autio-Gold, 2002). Activa BioACTIVE-Restorative is a resin-based flowable composite containing glass ionomer and resin composite components. An acid-base setting reaction occurs between the fluoroaluminum silicate particles and the polyacid components (Sauro et al., 2019). Activa has the ability to release and replenish calcium, phosphate and fluoride from saliva, thus stimulating the formation of apatite. This is effective against discoloration and microleakage, and improves mechanical properties (Gjorgievska et al., 2008; Firouzmandi et al., 2020). One of the most preferred methods for measuring microleakage is dye penetration with methylene blue due to ease of application and fair price. Methylene blue has a role in tracing the degree of infiltration and has lower molecular weight even smaller than bacteria thus detecting leakage where bacteria could not penetrate (Patel et al., 2015).

After the cavity preparation, the smear layer formed on the cavity and enamel-dentin border, and the microorganisms in the dentinal tubules cannot be eliminated completely (Akturk et al., 2019; Attiguppe et al., 2019; Cellik & Bahsi, 2019). For restorations longevity, the presence of bacteria plays a significant role in success of the treatment (Imazato et al., 2001). It has been shown that, various cariogenic microorganisms survive more than a year under restorative materials (Sharma et al., 2011). Cavity disinfection is an acceptable procedure that can prevent the risks resulting from the microorganisms in the tooth structure (Elkassas et al., 2014). One of the most common broad-spectrum antibacterial cavity disinfectant solutions, clorhedixine digluconate (CHX) (Varoni et al., 2012) is considered as the gold standard due to its potential to eliminate a wide range of gram-positive and gram-negative bacteria (Balagopal & Arjunkumar, 2013).

The purpose of this study was to evaluate the effect of CHX containing cavity disinfectant on microleakage of Class V self-adhesive resin-based composite restorations. The null hypothesis (H0) of the study is that there is no significant difference in microleakage amount between applications with or without cavity disinfectant.

MATERIALS AND METHODS

Ethics committee approval of this *in vitro* study was received by Ethics Committee of Marmara University Faculty of Dentistry with the number 2021-21 on the date of 07/10/2021. Forty non-beveled Class V cavities (4 mm height x 2 mm width x 2mm depth) 1 mm above the cemento-enamel junction were prepared on lingual and buccal surfaces of 20 molar teeth. were prepared on lingual and buccal surfaces of 20 molar teeth. In this study, 20 non-carious human molar teeth extracted for periodontal or orthodontic reasons were used and disinfected with 0.1% thymol solution. After disinfection protocol, all teeth were immersed in distilled water for 24 h. A total of 40 non-beveled Class V cavities (with dimensions of 4x2x2 mm) were prepared on both the buccal and lingual surfaces. The occlusal and gingival margins of the cavities

were located on the enamel. Samples were randomly divided into 4 groups (Fig. 1).

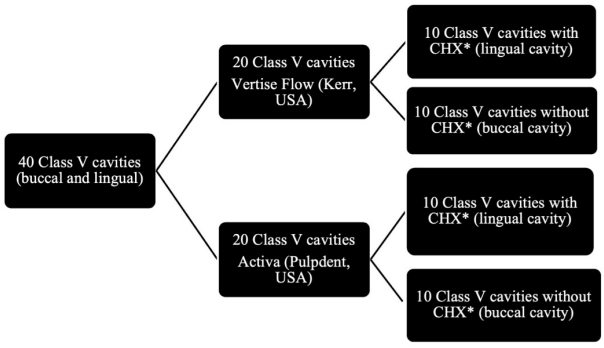

Figure 1: Study plan and groups. (% 2 CHX).

Following 37% orthophosphoric acid (Etching Gel, President, GERMANY) application to enamel for 15 s, each cavity was rinsed for 20 s and air-dried for 2 s avoiding excessive pressure. All the lingual cavities were disinfected by 2% CHX (Cavity Cleanser, BISCO, USA) application according to manufacturer's instructions. Buccal and lingual cavities were restored using 2 different self-adhesive composites (Vertise Flow, Kerr, USA; Activa BioACTIVE, Pulpdent, USA) as recommended by the manufacturers (Table 1). After 20 s polymerization with Valo Cordless (Ultradent, USA) LED curing light (power output: 1000 mW/cm2), finishing and polishing procedures of the restorations were performed using 4-step grinding (coarse, medium, fine, and ultrafine) aluminum oxide-coated discs (Finishing Discs, Bisco, USA) and polishing rubber (Enhance Pogo, Dentsply Sirona, USA). Each disc was used for only 5 samples, and the polishing time was 15 s for each disc for all the samples. The teeth were coated with a clear nail polish excluding the restoration surface area and aged in distilled water at room temperature (24°C) for 6 months. Samples were immersed in 2% methylene blue solution for one hour. Each tooth was then sectioned longitudinally in buccolingual direction with a diamond saw (Isomet 1000, Buehler, USA). Occlusal and gingival margin microleakage amounts were scored under x8 and x20 magnification using a stereomicroscope (Leica MZ7.5, Leica Microsystems, Germany) (Fig. 2). Scoring criteria for dye penetration indicating microleakage are listed in Table 2.

Table 1. The self-adhesive composites and cavity disinfectant, their compositions and manufacturer's instructions for use.

Product Name	Manufacture	Composition	Instructions for Use
Vertise flow	Kerr, USA	GPDM adhesive monomer, Prepolymerized filler containing barium glass filler, nano-sized colloidal silica, nano-sized ytterbium fluoride	Vertise flow composite is self-adhesive and therefore does not require etching or bonding protocol prior to placement. Wash thoroughly with water spray and air dry at maximum air pressure for 5 s. Select the desired shade. Dispense Vertise flow onto prep with provided dispensing tip. Use provided brush to apply Vertise flow to the entire cavity wall and beveled area with moderate pressure for 15-20 s to obtain a thin layer (<0.5mm). Remove excess material around margins with the brush if necessary. Light cure for 20 s. For A3.5 and Universal Opaque shades, light cure each increment for 40 s.
Activa BioACTIVE	Pulpdent, USA	Bioactive glass, silica, diurethane modified with hydrogenated polybutadiene, methacrylate monomers, modified polyacrylic acid, sodiumfluoride, camphorquinone (photoinitiator)	Isolate and prepare tooth to receive a restoration. Ideal margin preparations are rounded with no sharp angles. Etch prepared surface for 10-15 s with 37% phosphoric acid etching gel, or selective etch enamel for 15 s, rinse and lightly dry, removing all excess moisture with high volume evacuation, compressed air, and/or a cotton pellet, but do not desiccate the tooth. Place mix tip at cavity floor. Apply ACTIVA in increments of up to 4 mm, keeping mix tip submerged in the material. Light cure for 20 s (with low intensity setting) between each layer.
Cavity Cleanser	BISCO, EUA	2% CHX	Apply acid according to your choice of adhesive. A dry, but non-desiccated, surface is ideal before applying. Moisten dentin surface with CAVITY CLEANSER using a brush or absorbent pellet. Remove puddled solution with a new absorbent pellet, leaving site moist. Do not dry. Continue with adhesive and direct composite technique.
Etching Gel	President, GERMANY	37% Phosphoric acid – Purified water – Thickener – Colorant	Isolate tooth and prepare the cavity in a conservative manner. Equip the disposable tip after removing the cap. Etch enamel and dentin with etching agent for 15 s. Rinse gel thoroughly and remove excess water from the preparation with a gentle stream of air for 1-2 s.

Table 2. Occlusal and gingival margin microleakage scores.

Score	Scoring criteria for dye penetration indicating microleakage (occlusal margin)	Scoring criteria for dye penetration indicating microleakage (gingival margin)
0	No dye penetration	No dye penetration
1	Dye penetration limited to ½ or less of the occlusal wall	Dye penetration up to ½ of the gingival wall
2	Dye penetration exceeding ½ of the occlusal wall	Dye penetration along the gingival wall
3	Dye penetration limited to ½ of the cavity base	Dye penetration up to ½ of the cavity base
4	Dye penetration exceeding ½ of the cavity base	Dye penetration exceeding ½ of the cavity base

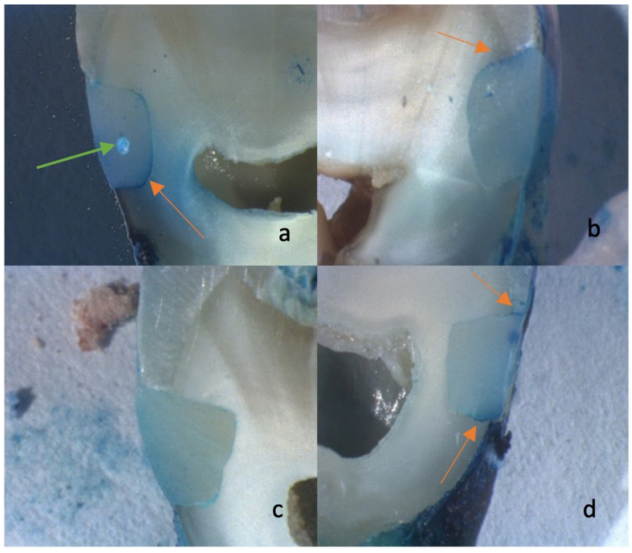

Figure 2: Microleakage evaluation of the restorations under the stereomicroscope. a – Gap in composite (green arrow) and gingival margin score as 3 (orange arrow), b – Occlusal margin score as 2, c – Occlusal and gingival margin scores as 0, d – Occlusal margin and gingival margin scores as 1.

The obtained data were analyzed using IBM SPSS V23 (IBM Corp, USA). Analysis results are presented as frequency (percentage) for categorical data. Chi-square test was used to compare microleakage amounts according to different restorative materials and CHX application. The significance level was set at $p<0.05$.

RESULTS

The microleakage scores obtained using stereomicroscope are presented in Table 3 and 4. No statistically significant difference was detected between the microleakage amounts of the restorations regarding CHX application ($p>0.05$). Composite type did not have any statistically significant effect between the microleakage amounts in CHX applied groups. Similarly, CHX application did not have any significant effect between the microleakage amounts in the restorations (Table 3).

Table 3. Comparison of microleakage in Class V cavities compared to CHX application.

Cavity	Composite	Microleakage Score	CHX (-)	CHX (+)	Total	p*
Oclusal	Vertise Flow	Score 0	2(20)	1(10)	3(15)	0.819
		Score 1	7(70)	8(80)	15(75)	
		Score 2	1(10)	1(10)	2(10)	
		Score 4	0(0)	0(0)	0(0)	
	Activa BioActive	Score 0	2(20)	2(20)	4(20)	0.587
		Score 1	7(70)	8(80)	15(75)	
		Score 2	0(0)	0(0)	0(0)	
		Score 4	1(10)	0(0)	1(5)	
	Total	Score 0	4(20)	3(15)	7(17.5)	0.735
		Score 1	14(70)	16(80)	30(75)	
		Score 2	1(5)	1(5)	2(5)	
		Score 4	1(5)	0(0)	1(2.5)	
Gingival	Vertise Flow	Score 0	4(40)	6(60)	10(50)	0.638
		Score 1	5(50	3(30)	8(40)	
		Score 2	1(10)	1(10)	2(10)	
		Score 4	0(0)	0(0)	0(0)	
	Activa BioActive	Score 0	3(30)	1(10)	4(20)	0.557
		Score 1	6(60)	7(70)	13(65)	
		Score 2	0(0)	1(10)	1(5)	
		Score 4	1(10)	1(10)	2(10)	
	Total	Score 0	7(35)	7(35)	14(35)	0.944
		Score 1	11(55)	10(50)	21(52.5)	
		Score 2	1(5)	2(10)	3(7.5)	
		Score 4	1(5)	1(5)	2(5)	
Total	Vertise Flow	Score 0	6(30)	7(35)	13(32.5)	0.942
		Score 1	12(60)	11(55)	23(57.5)	
		Score 2	2(10)	2(10)	4(10)	
		Score 4	0(0)	0(0)	0(0)	
	Activa BioActive	Score 0	5(25)	3(15)	8(20)	0.577
		Score 1	13(65)	15(75)	28(70)	
		Score 2	0(0)	1(5)	1(2.5)	
		Score 4	2(10)	1(5)	3(7.5)	
	Total	Score 0	11(27.5)	10(25)	21(26.3)	0.896
		Score 1	25(62.5)	26(65)	51(63.7)	
		Score 2	2(5)	3(7.5)	5(6.3)	
		Score 4	2(5)	1(2.5)	3(3.8)	

There was no statistically significant difference between the distribution of microleakage scores of the restorations considering different brands of self-adhesive composites. Likewise, there was no statistically significant difference between the distributions of microleakage occurring in the restoration according to the use of different brands

of self-adhesive composites (Table 4). The amount of microleakage has not been scored as 3 in any of the groups. There is no statistically significant difference between the distributions of microleakage scores of the occlusal and gingival margins (Table 5).

Table 4. Comparison of microleakage in restoration according to the use of different brands of self-adhesive composites in class V cavities.

Cavity	Microleakage Score	Vertise Flow	Activa BioActive	Total	p*
Occlusal	Score 0	3 (15)	4 (20)	7 (17.5)	0.37
	Score 1	15 (75)	15 (75)	30 (75)	
	Score 2	2 (10)	0 (0)	2 (5)	
	Score 4	0 (0)	1 (5)	1 (2.5)	
Gingival	Score 0	10 (50)	4 (20)	14 (35)	0.107
	Score 1	8 (40)	13 (65)	21 (52.5)	
	Score 2	2 (10)	1 (5)	3 (7.5)	
	Score 4	0 (0)	2 (10)	2 (5)	
Total	Score 0	23 (57.5)	28 (70)	51 (63.7)	0.09
	Score 1	13 (32.5)	8 (20)	21 (26.3)	
	Score 2	4 (10)	1 (2.5)	5 (6.3)	
	Score 4	0 (0)	3 (7.5)	3 (3.8)	

Table 5. Comparison of microleakage with respect to the occlusal and gingival margins.

Microleakage score	Occlusal	Gingival	Total	p*
Score 0	7 (17.5)	14 (35)	21 (26.3)	0.216
Score 1	30 (75)	21 (52.5)	51 (63.7)	
Score 2	2 (5)	3 (7.5)	5 (6.3)	
Score 4	1 (2.5)	2 (5)	3 (3.8)	

DISCUSSION

Microleakage has been defined as one of the important factors causing failure of resin-based composite restorations (Guo et al., 2016). The ability of a composite to reduce the amount of microleakage at tooth-restoration interface is also a basic factor in estimating its clinical success (Siso et al., 2009). This study was designed to compare the microleakage properties of CHX containing cavity disinfectant and self-adhesive flowable composite to analyze the relationship between resin-dental tissue and microleakage.

Siso et al. evaluated microleakage in composite resin restorations following antimicrobial pretreatments such as laser, CHX, adhesive agent, and scores were lower at enamel margins than gingival margins (Siso et al., 2009). In the present study, there was no statistically significant difference between the occlusal and gingival microleakage scores. This result may be due to the fact that the occlusal and gingival margins of the cavities were located 1 mm above the cemento-enamel junction. Low ow surface energy, high organic components, tubular structure, and dentinal fluid pressure make bonding to dentin more difficult than enamel (Van Ende et al., 2017).

In their study based on a 6 to 12 months evaluation, Angeloni et al. showed that CHX had no effect on bonding to dentin in a self-adhesive restoration (Araujo et al., 2001). In the current study, CHX application had no effect on microleakage at the cavity margins at the enamel level. However, they said that there was a significant difference in the storage time for bond strength. In the present study, there was only 6 months of storage time, which can be considered a limitation

CHX application did not have any significant effect on the adhesion of the restorative material to the dental tissues. In Activa BioActive and Vertise Flow self-adhesive flowable composites, no significant difference was found between the microleakage values regardless of CHX application. There are studies reporting that self-adhesive flowable composites have similar properties such as fracture strength when compared with each other (Firouzmandi et al., 2020). Only microleakage was evaluated in the present study and selective etching was performed for both composites and no significant difference was observed.

An ideal disinfectant should have an effective antimicrobial role and should not inhibit the adhesion of the restorative material (Elkassas et al., 2014). The results of this *in vitro* study showed that the use of a 2% CHX-containing cavity cleaning solution before application of self-adhesive composites had no effect on the sealing ability. There was no statistically significant difference for any group in the microleakage assessment for the restorations preconditioned with CHX.

The results of this *in vitro* study support the null hypothesis that microleakage is not affected by disinfectant use. Further studies with standardized protocols are required to allow robust conclusions regarding microleakage and biocompatible restorations. The effect of cavity disinfectant on self-adhesive flowable composite should also be evaluated since they can reduce the clinical steps of great importance in restorative dentistry.

Correlating the results of this study with the available literature revealed that CHX application for cavity disinfection had no effect on the bonding ability of self-adhesive flowable composite restorations. In addition, further *in vitro* and *in vivo* studies are required to assess the interaction and long-term clinical success of CHX with other self-etch adhesive systems.

CONCLUSION

Within the limitations of this *in vitro* study:

1. CHX pretreatment had no effect on microleakage in Class V cavities restored with self-adhesive flowable composite resins.

2. Self-adhesive flowable composites showed no difference in microleakage.

3. Microleakage amount at the occlusal and gingival margins of the cavities was similar.

Conflict of Interests

N/A.

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How to cite this article: Kaya BD, Acar E, Farshidian N, Göçmen GB, Atalı PY, Tarçın B. The Effect of Cavity Disinfectant on Microleakage of Self-adhesive Composite Restorations in Class V Cavities. European Journal of Research in Dentistry, 2023;7(3): 115-121. DOI: http://dx.doi.org/10.29228/erd.55