SINIF II RESTORASYONLARDA KAVİTE DEZENFEKTANLARININ MİKROSIZINTIYA ETKİSİNİN ELEKTRON MİKROSKOBU (SEM) İLE DEĞERLENDİRİLMESİ

THE EVALUATION OF EFFECT OF CAVITY DISINFECTANTS ON MICROLEAKAGE IN CLASS II RESTORATIONS UNDER SCANNING ELECTRON MICROSCOPE (SEM)

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Özet

Bu çalışmanın amacı, klorheksidin jel kavite dezenfektanın sınıf II kavitelerde self-etching adeziv sistemlerde mikrosızıntı üzerine etkisini değerlendirmekti.

Çalışmada ortodontik ve periodontal nedenlerle çekilmiş 90 adet küçük azı dişine standart sınıf II kaviteleri açıldı. Dişler, her grupta 15 kavite olacak şekilde rastgele 6 gruba ayrıldı: ***Grup 1:*** Sadece Self-etch (Xeno V) adeziv ajan, ***grup 2:*** Chx jel 20 sn ve self-etch adeziv (Xeno V) ***grup 3:*** Sadece Self-etch (Clearfil S^3 Bond) adeziv ajan, ***grup 4:*** Chx jel 20 sn ve self-etch adeziv (Clearfil S^3 Bond), ***grup 5:*** Sadece Self-etch (G-bond) adeziv ajan, ***grup 6:*** Chx jel 20 sn ve self-etch adeziv (G-bond) uygulandı, daha sonra tabakalama yöntemiyle kompozit restorasyonlar yapıldı. Bütün örnekler 10.000 kez termal siklus işleminden sonra, 24 saat % 0.5'lik metilen mavisi içerisinde bekletildi. Dişler mesio-distal yönde kesildi, mine ve gingival duvarlar 0 ile 4 arasındaki skorlar ile değerlendirildi. Ayrıca, her bir gruptan rastgele 6 örnek seçildi ve rezin-diş sert dokusu ara yüzeyi SEM altında incelendi. İstatistiksel olarak Kruskal Wallis, tek yönlü varyans analizi ve Tukey testleri kullanıldı.

Kavite dezenfektanı uygulanmayan gruplar arasında en az gingival ve oklüzal mikrosızıntı skorları grup 3'de görülmekle beraber, grup 1 ve grup 5 arasında istatistiksel olarak fark görülmemiştir. Kavite dezenfektanı uygulanan gruplarda hem gingival hem de oklüzalde istatistiksel olarak fark bulunmamıştır. Kavite dezenfektanları uygulanan ve uygulanmayan gruplar arasında istatistiksel olarak fark bulunmamıştır.

Sınıf II kavitelerde klorheksidin jel kavite dezenfektanı olarak uygulanması self-etching adeziv sistemlerde mikrosızıntı üzerine olumsuz bir etki göstermediği tespit edildi.

Anahtar Sözcükler: Mikrosızıntı, Kavite dezenfektanı, Sınıf II kavite, Elektron mikroskobu (SEM), self-etch adeziv sistem, klorheksidin jel, termal siklüs, adeziv sistemler, posterior kompozit.

Abstract

The aim of this study was to evaluate the effect of chlorhexidine gel on microleakage of self-etch adhesive systems in class II cavities.

Ninety standard class II cavities were prepared on extracted premolar teeth. Teeth were randomly divided into six groups of 15 specimens each. ***Group 1:*** Xeno V was applied, with no cavity disinfectant. ***Group 2:*** CHX gel was applied for 20 s, followed by Xeno V. ***Group 3***: Clearfil S^3 Bond was applied, with no cavity disinfectant. ***Group 4:*** CHX gel was applied for 20 s, followed by Clearfil S^3 Bond. ***Group 5:*** G Bond was applied, again with no cavity disinfectant. ***Group 6:*** CHX gel was applied for 20 s, followed by G Bond. All cavities were restored with composite, immersed in distilled water for 24 h, thermocycled 10,000 times, immersed in 0.5% methylene blue for 24 h. Teeth were mesio-distally sectioned; enamel, gingival margins were evaluated and scored from 0 to 4. Six specimens from each group were randomly selected for resin-hard tissue interface examination under SEM. All data were statistically analyzed using Kruskal-Wallis and the Tukey test.

Among non-disinfectant groups, group 3 showed the lowest occlusal and gingival microleakage score, no significance was seen between groups 1 and 5. In cavity disinfectant groups, no significance was determined in occlusal and gingival microleakage scores. No significance was determined between the cavity disinfectant and non-disinfectant groups.

The application of chlorhexidine gel in class II cavity restorations has no negative effect on microleakage.

Key words: Microleakage, cavity disinfectant, class II cavities, scanning electron microscope (SEM), chlorhexidine gel.

Introduction

The aim in restorative dentistry is to select the ideal material for the purpose of gaining good the esthetic, phonetic and functional deficiencies caused by loss of dental structures. Since the materials applied come into contact with soft tissues and fluids in the oral environment, biological compatibility has to be considered in addition to mechanical and physical properties. Compatibility between dental tissue and restorative materials is of critical importance if restorations are to maintain their performances in the long term (1). Weak bonding of restorative material to the dental tissue is one of the main causes of microleakage (2).

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Since they are esthetic and economical, composite restorations are widely preferred in class II cavities (3). Restoration success depends on the dentin bonding systems applied. These are materials developed in order to establish adhesion between the composite and dentin(4). These materials prevent microleakage, marginal discoloration, secondary caries and resulting pulpal reactions by hermetically sealing the surface between the cavity wall and restorative material. In addition, they reinforce weakened dental tissues by reducing functional stresses acting on the tooth and restorative materials (5).

Clinical success in adhesive procedures depends on the bonding system and technique used (6). Bonding systems are materials consisting of monomer solutions and bond the restorative material and dental tissue through monomer polymerization. As adhesive bonding between the two surfaces develops, adhesion takes place between more complex structures such as enamel-dentin-bond system-composite, porcelain and amalgam (7).

Dentin bonding systems have been classified in different ways in previous studies. Of these, the classification described by Van Meerbeek et al.(8) in 2001 today enjoys wide scientific acceptance. This is based on the number of steps involved in the application of dentin adhesive agents and on dentin and enamel bonding strategies. The classification is divided into three groups, etch and rinse bonding systems applied with acid etching and rinsing, self-etch bonding systems and glass ionomer bonding systems.

In etch and rinse bonding systems, the enamel and dentin surface is etched with a 30%-40% phosphoric acid concentration and then rinsed. In this way, the smear layer is completely cleared after etching and the superficial hydroxyapatite crystals are demineralized. In traditional etch & rinse systems, priming and bonding are performed following the etching process (9). Two-step etch and rinse bonding systems were developed in order to reduce and simplify the stages involved. In these systems the priming and adhesive steps are performed in a single stage, and these are known as one bottle systems. Both two- and three-step adhesives adhere to the dental tissues through the same mechanisms. In the etching stage, the phosphoric acid removes the smear layer, while at the same time exposing the dentin and collagen fibril network to a depth of 3-5 μM. These exposed collagen fibrils constitute a micro-adhesion network, giving rise to micromechanical bonding with the diffusion of the resin over the network (10). Surface roughening and application of acid to the enamel and dentin surface and penetration into the resulting microporous structure of a high flow monomer structure (bonding system) represents the essential mechanism in the adherence of resin-based filling materials to the tooth (8).

Self-etch bonding systems, which themselves roughen the dental surface, were developed as an alternative to total etch systems in order to reduce technical clinical sensitivity and duration of application. These dentin bonding systems require no separate etching and rinsing step. In this way, the length of application is shortened, while technical sensitivity and mistakes during application are reduced. These self-etch systems demonstrate their effects by dissolving (11) or modifying (12) the smear layer with acidic monomers. The acidic monomers in the structure of self-etch systems are able to roughen the surface as they etch the enamel and dentin and enter into a reaction on the surface of the dentin layer by penetrating down through the resin smear layer. As a result, the dissolved smear layer and demineralization products are rinsed away, as in etch and rinse systems, and these residues conjugate with the adhesive resin (13). The smear layer is thus modified through monomer infiltration, allowing it to combine with the hybrid layer, and thus becomes an element of the adhesion interspace.

Self-etch bonding systems may be classified in two ways. These are known as one- and two-step self-etch adhesive systems, depending on method of application. In two-step systems, hydrophobic resin is applied after the primer containing acidic monomers, while in one-step systems, the acid monomer and bonding resin come in one bottle, meaning that application is reduced to a single step (14).

Depending on their pH values, self-etch bonding systems are also classified as strong (pH≤1), medium (pH≈1.5) or weak (pH≥2). Self-etch bonding systems contain self-etch adhesive monomers, transverse bounded monomers and monofunctional monomers. Self-etch adhesive monomers include carboxylic, phosphoric or dehydrogenphosphate acid groups. Thanks to these groups, self-etch bonding systems provide roughening in dental hard tissues. With the monomers that they contain, they also establish chemical bonding by performing chelation with the inorganic component of dental hard tissues. Water is necessary for ionic bonding, and self-etch bonding systems are therefore generally water-based. Assistant solvents such as acetone and ethanol may also be incorporated in these systems(15).

Glass ionomer bonding systems are the only material capable of bonding to the enamel and dentin tissue with no surface preparation to these. Surface preparation with weak polyalcaenoic acid has also been reported to increase adhesion strength significantly. Glass ionomer bonding is achieved through a one- or two-step application. The use of short-term polyalcaenoic acid cleans the surface, removes the smear layer and exposes the collagen fibrils to a depth of 0.5-1 μm. The glass ionomer compounds then diffuse to this region and micromechanical bonding results. Chemical adhesion also occurs between the carboxyl groups and the hydroxyapatite Ca ions, and these thus adhere to the collagen fibrils. The main difference between resin based self-etch systems and glass ionomers is that glass ionomers are self-etch systems including polycarboxylic based polymers with a higher molecular weight. This characteristic limits glass ions' infiltration capacities and only a very thin hybrid layer forms (17).

Studies have reported that microleakage is a significant factor in postoperative sensitivity, secondary decay, pulpal necrosis and pulpal inflammation (17,18,19). Postoperative sensitivity and secondary decay play a major role among the reasons for restoring composite resin again (18,20). Therefore, it is of considerable importance, once the decayed dentin has been removed from the cavity floor and walls, for any residual bacteria left in the smear layer, in the enamel dentin interface region or the dentin tubules to be eliminated (21).

Cavity disinfectants (22), acid preparates (23,25), antibacterial materials (26) and laser light are recommended in order to eliminate bacteria and prevent the effects they give rise to; the use of flowable composite bases has been recommended to reduce polymerization-shrinkage associated cavities and to ensure the composite is better adapted to the cavity (27).

Studies on the subject have recommended the use of restorative materials with antibacterial properties or cavity disinfectants in order to prevent residual decay of bacterial origin (28,30). Researchers have tested several chemical substances containing iodine, hydrogen peroxide, EDTA, sodium hypochlorite (NaOCl) and chlorhexidine digluconate as cavity disinfectants over the last 20 years (31,32). Chlorhexidine is an antibacterial that has been widely used in dentistry, as well as medicine, since the 1970s (33).

This agent affects bacterial metabolic activity and is bacteriostatic at low concentrations while it plays a bactericidal role irreversibly precipitating the cellular content at high concentrations (34).

Chlorhexidine reduces the levels of certain sensitive (chlorhexidine-sensitive) micro-organisms, particularly *Streptococcus mutans* (35). Chlorhexidine comes in toothpaste form (0.4%), as a mouthwash (0.12% and 0,2%), a gel (1%) and as a varnish (1%, 10%, 20% and 35%)(33).

This study evaluated the effect of chlorhexidine gel cavity disinfectant on microleakage in class II cavities by comparing self-etch adhesive systems.

Materials And Methods

Ninety decay-free premolar teeth were used. Post-extraction, tissues above the roots were removed using a scaler, and then were stored in saline solution at room temperature. Each tooth was sectioned mesio-distally by a single researcher, and standard class II cavities were prepared with a diamond burr (835/008-3 ML, Diatech Dental AG, Heerbrugg) under water cooling in such a way as the gingival margin was 1 mm above the enamel-dentine margin. The occlusal width of the cavity was 1/3 of the distance between the tubules while the width in the proximal region was 1/3 of the bucco-lingual distance, and cavity depth was prepared so as to extend beneath the enamel-dentine margin. Care was taken that the gingival margin should be of such a width as to include both enamel and dentin. No beveling was applied to the cavities' enamel margins.

Teeth were then randomly assigned into groups of 15 teeth each:

Group 1: Self-etch (Xeno V) adhesive was applied. This group was taken as the control group. No cavity disinfectant was applied to the class II cavity surface. A self-etch bonding system (Xeno V- Dentsply DeTrey, Konstanz, Germany) was applied to the dentin surfaces. Excess solvent was removed using an air spray and polymerization was performed using an LED (Light Emitting Diode -Elipar Freelight, 3M ESPE, Germany) light source for 10 s at a power of 1000 mW/cm2. Quixfill posterior composite (Xeno V- Dentsply DeTrey, Konstanz, Germany) was then applied as restorative material and polymerized for 20 s with an LED.

Group 2: Following the application of CHX gel for 20 s, self-etch adhesive (Xeno V) and Quxfill posterior composite (Xeno V-Dentsply De Trey, Konstanz, Germany) were applied as in group I.

Group 3: Following the preparation of class II cavities, an adhesive system (Clearfil S^3 Bond-Kuraray Dental, Izmir, Turkey) was applied and maintained for 20 s, after which it was dried under pressurized air for 5 s and light polymerized for 20 s with an LED (Light Emitting Diode -Elipar Freelight, 3M ESPE, Germany). Composite (Clearfıl Photo Posterior-Kuraray Dental Izmir, Turkey) was used in line with the manufacturer's recommendations.

Group 4: CHX gel was applied for 20 s, after self-etch adhesive (Clearfil S^3 Bond) and composite were applied as in group 3.

Group 5: Once the class II cavity had been prepared self-etch (G bond-GC Corporation, Tokyo, Japan) adhesive was applied for 30 s and left for 20 s to apply a single layer. It was then dried for 5 s under pressurized air and light polymerized for 20 s using an LED (Light Emitting Diode -Elipar Freelight, 3M ESPE, Germany). Composite (Gradio direct posterior-Kuraray Dental Izmir, Turkey) was applied in line with the manufacturer's recommendations.

Group 6: CHX gel was applied for 20 s, after which G-bond adhesive and composite restoration were applied as in group 5.

Once all restorations had been completed, finishing and polishing were performed under water cooling with the help of fine grain diamond friezes and aluminum oxide coated disks (Sof-Lex, 3M ESPE, St. Paul, MN, USA). Following the finishing and polishing procedures, teeth were first kept in a stove for 24 h at 37 ºC and 100% humidity.

Teeth were then thermocycled 10,000 times for 30 s at 5 ± 2 ºC and 55 ± 2 ºC. Two layers of nail polish were applied to the surfaces of all teeth, in such a way that areas up to 1 mm from restorations would remain exposed. Teeth were then kept for 24 h in a 0.5% methylene solution in a stove at 37 ˚C. In order to be able to investigate microleakage, teeth were divided into two, vertically, along the mesio-distal aspect under water cooling with the help of a 0.2 mm diamond separator (Isomet, Buehler Ltd, Lake Bluff, IL, USA). Dye infiltration at the cavity margins was scored under x14 magnification under a stereo microscope (Olympus Co., Tokyo, Japan) (Figures 1-6). Specimens given different scores by the two observers were again evaluated by both together and a single score eventually agreed and recorded for each specimen.

Microleakage scoring in class II cavities was performed as follows (36) (Figure 1):

0 = No dye leakage

1 = Dye leakage in up to half of the cavity wall

2 = Dye leakage in all of the cavity wall

3 = Dye leakage in the cavity walls and cavity roof

4= Dye leakage partly or completely extending to the pulp

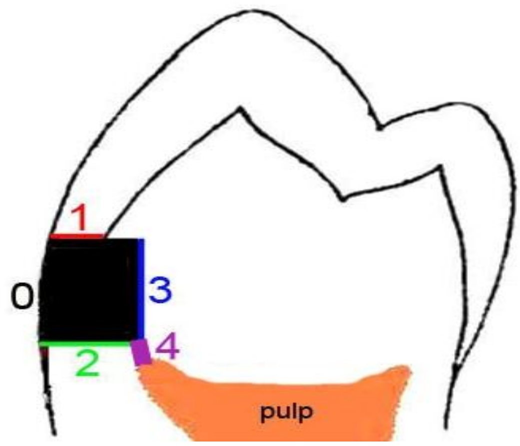

Figure 1: Analysis of microleakage scores

Six teeth were selected at random from each group in order to examine the hard tissue

tissue-restoration interface under a scanning electron microscope (SEM (JSM-5300; JEOL, Tokyo, Japan). Before analysis under SEM, specimens were kept in 6 N (6 mol/l) HCl for 5 s, and then for 5 min in 5% hydrogen peroxide and coated with Au-Pd in an ion coating unit (SEM Coating Unit E 500, POLARON Equipment Limited, Barcelona, Spain).

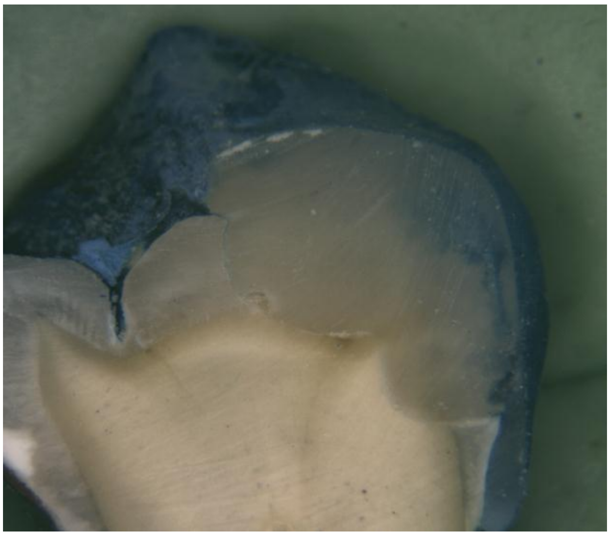

Figure 2. Group 1 (Xeno V and Quxfill posterior composite) self-etch adhesive composite restoration group gingival and occlusal microleakage value =0 (x15)

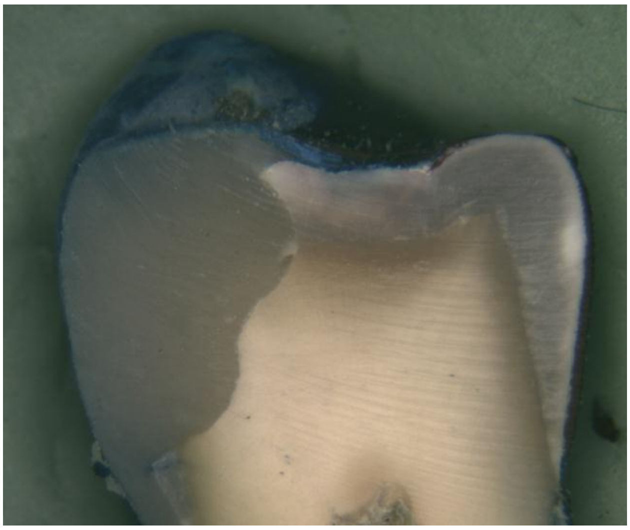

Figure 3: Group 2, CHX gel Xeno V and Quixfill posterior composite restoration group gingival and occlusal microleakage value =0 (x15)

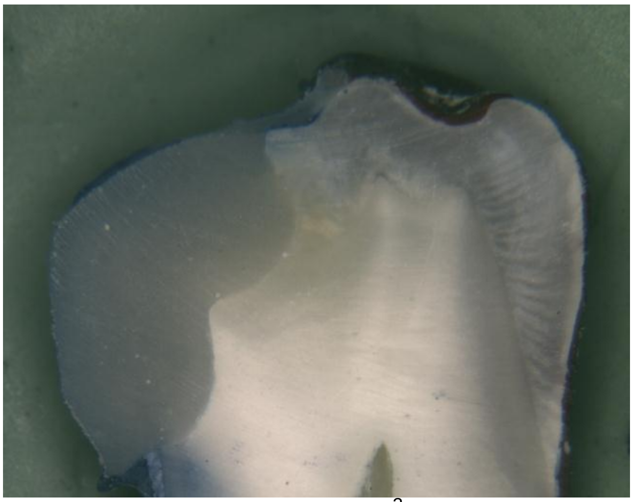

Figure 4: Group 3, Clearfil S^3 Bond and photo posterior composite restoration group gingival and occlusal microleakage value =0 (x15)

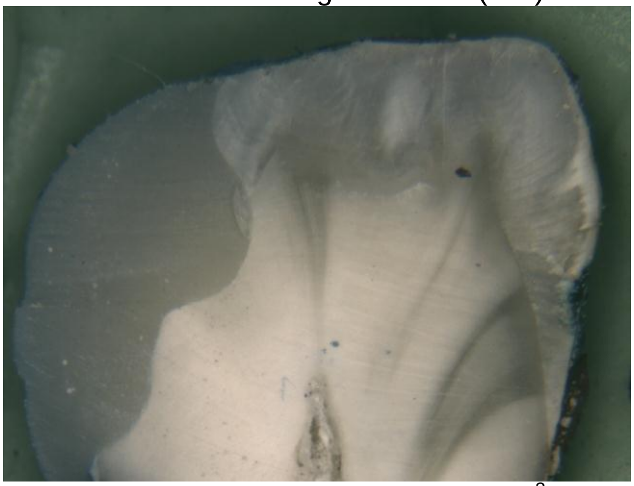

Figure 5: Group 4, CHX gel Clearfil S^3 Bond and photo posterior composite restoration group gingival and occlusal microleakage value =0 (x15)

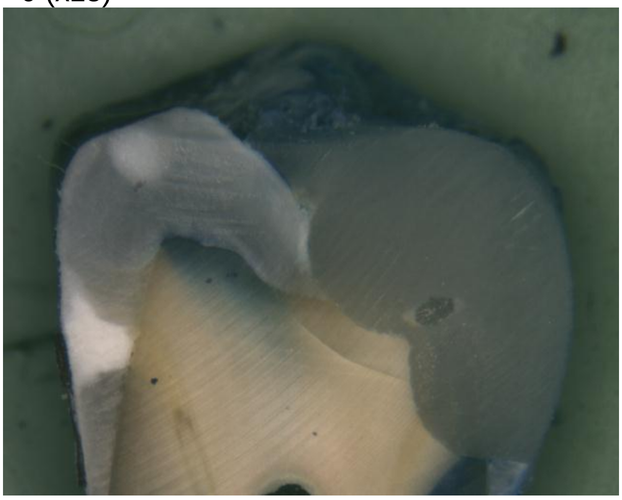

Figure 6: Group 5 G bond and gradia direct posterior composite restoration group gingival and occlusal microleakage value =0 (x15)

They were subsequently photographed once the surface morphology (hybrid layer, adhesive layer, tag and gaps) between the tooth hard tissue and restorative material had been examined under SEM (Figures 7-13).

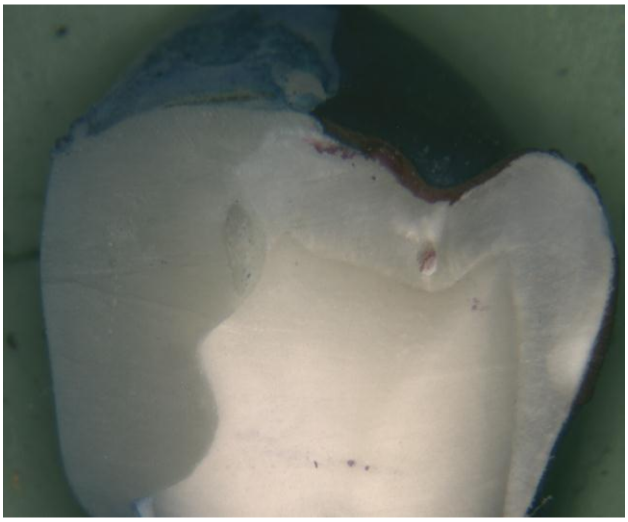

Figure 7: Group 6, CHX gel G-bond and gradia direct posterior composite restoration group gingival and occlusal microleakage value =0 (x15)

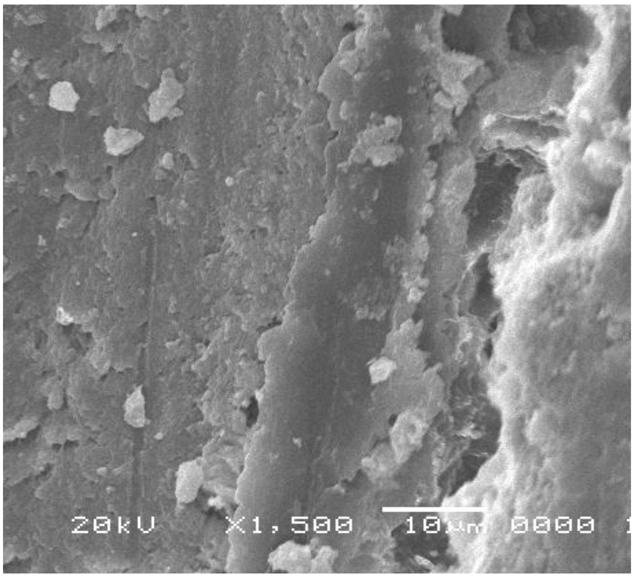

Figure 8: Group 1 SEM image (1500X)

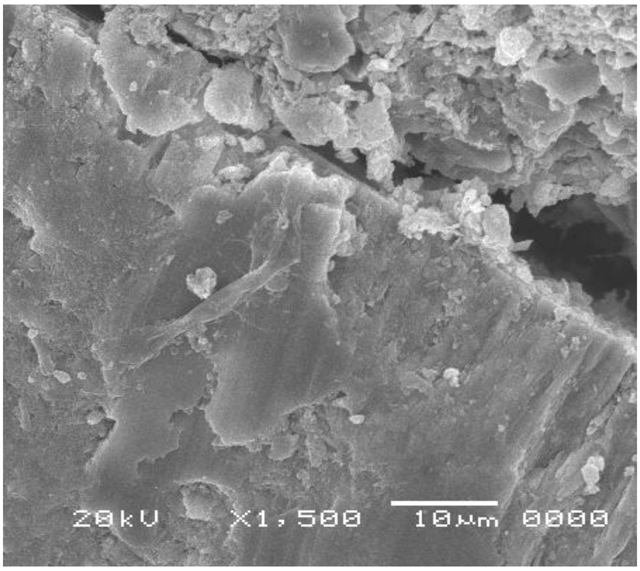

Figure 9: Group 2 SEM image (1500X)

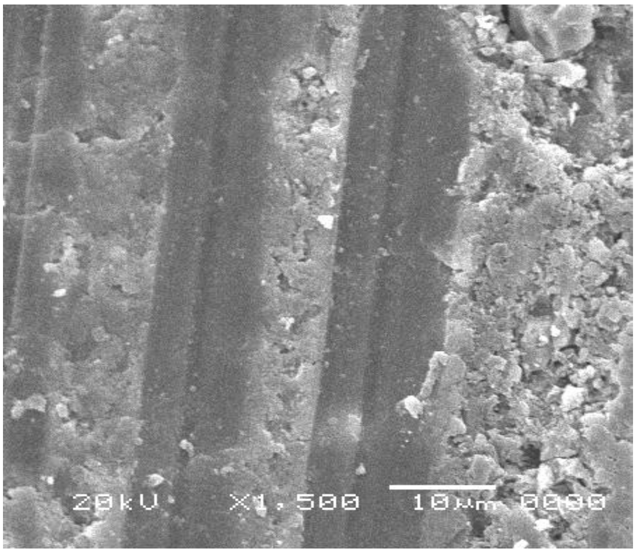

Figure 10: Group 3 SEM image (1500X)

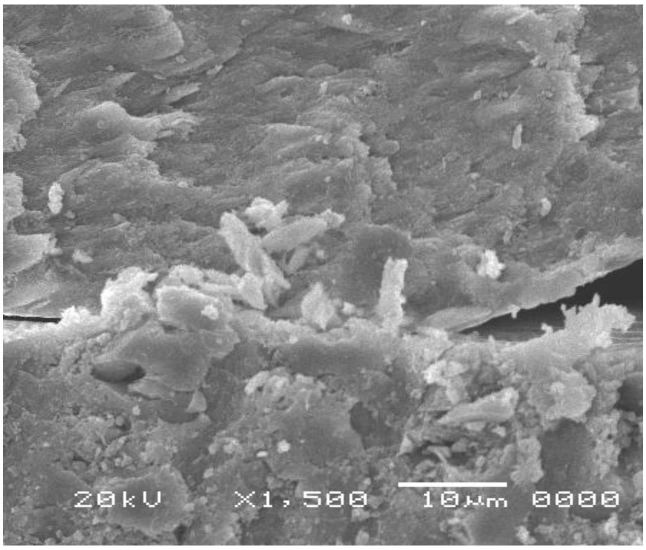

Figure 11: Group 4 SEM image (1500X)

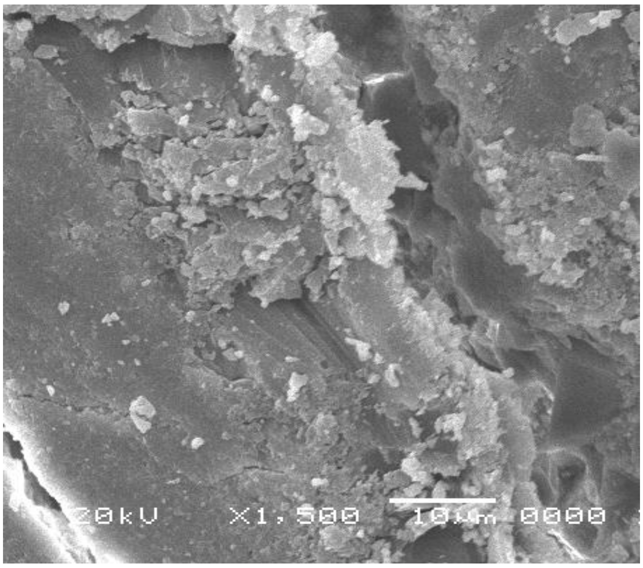

Figure 12: Group 5 SEM image (1500X)

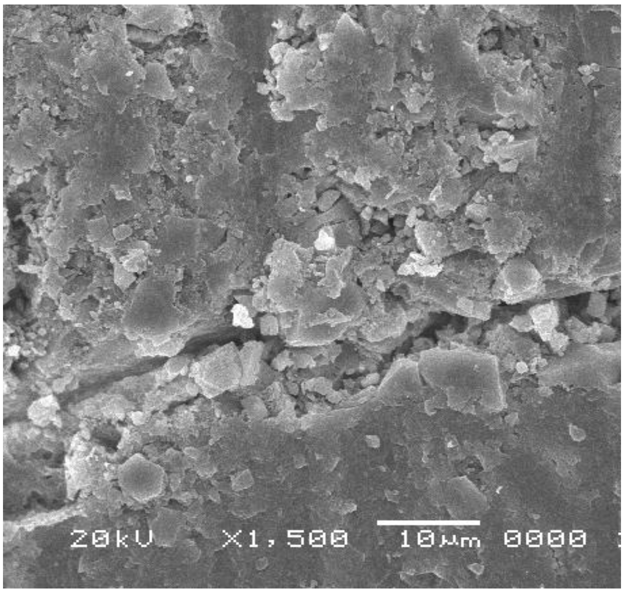

Figure 13: Group 6 SEM image (1500X)

Results

Microleakage scores on the basis of the margins and adhesive systems and restorative materials used in the study are shown in Table 1. Kruskal-Wallis test results showed no significant differences in microleakage values in the different cavity disinfectant groups (p>0.05)

Less microleakage was observed in the groups with no disinfectant procedure compared to those with disinfectant (p<0.05). Greater microleakage was recorded in the gingival margins compared to the enamel in all groups (p<0.05). At analysis of restorations' occlusal regions, the lowest microleakage was observed in group 3, in which no disinfectant was applied, while no difference was determined between groups 1 and 3, the other groups in which no disinfectant was employed (p>0.05) (Table 1). There was no difference between the groups in terms of gingival margins (p>0.05).

		Microleakage values				
		0	1	2	3	4
Group 1	gingival	9	3	2	1	0
	occlusal	11	2	2	0	0
Group 2	gingival	8	3	2	1	1
	occlusal	9	3	2	1	0
Group 3	gingival	11	2	2	0	0
	occlusal	12	2	1	0	0
Group 4	gingival	9	3	2	1	0
	occlusal	10	2	3	0	1
Group 5	gingival	10	2	0	2	1
	occlusal	11	2	1	1	0
Group 6	gingival	8	1	2	3	1
	occlusal	9	2	1	3	1

Table 1: Occlusal and gingival microleakage scores for all groups

No statistical difference was determined between the three different bonding systems at comparison of the gingival margins (p>0.05). No statistically significant difference was also established between gingival and occlusal values in any group. Although greater microleakage was observed in the groups in which no disinfectant was applied, the difference was not statistically significant.

Discussion

With the greater acceptance of conservative cavity preparations in modern dental practice, the importance of minimally invasive technique has increased and the sphere of use of resin-based restorative materials has expanded (35). In addition, residual bacteria in the cavity and secondary decay arising as a result of inadequate bonding between restorative material and tooth may lead to treatment failure (29, 32, 37).

Since patients' esthetic expectations and demands are increasing by the day, adhesive technology has also become more important. Adhesive has to bind the composite resins to the dental tissue very well in order for restorative dental treatment to be successful (38,39). Self-etch systems available on the market for that purpose are adhesives in which the acid etching and cleaning stages are eliminated and the likelihood of error during manipulation reduced. In addition to application procedures being shorter and simpler compared to conventional techniques, another major advantage with these systems is that they permit demineralization and resin infiltration (9, 10). Self-etching adhesives were used for these reasons in our study.

More reliable systems have emerged with advances in dentin adhesive formulae and application techniques. However, no material or technique that completely prevents microleakage has yet been discovered (40). Bonding strength, wettening properties, solvent structure and application characteristics are the determining factors in preventing microleakage in terms of dentin adhesive systems, while with composite resins these factors are elasticity module and shrinkage and thermal expansion coefficients (41).

The use of bonding agents and restorative materials, etching preparates and cavity disinfectants has been recommended in order to prevent postoperative sensitivity, secondary decay and pulpal inflammation associated with inadequate bonding and ensure elimination of bacteria (28, 32, 42).

Cavity disinfectants have been thought to have a negative effect on dentin bonding(21,31), although many studies have determined that cavity disinfectants have no effect on bonding and no negative impact on marginal leakage(28,32-34). Ercan et al.(32) applied hydrogen peroxide, and gel forms of chlorhexidine 0.2% and 2% as cavity disinfectants in an in vitro study and reported that bonding is not negatively affected in self-etching adhesive systems. Çelik et al.(29) showed that the effect of cavity disinfectants containing chlorhexidine on marginal leakage in class V restorations was not statistically significant. In the light of our results, the use of chlorhexidine as a cavity disinfectant in class II cavities has no effect on microleakage in self-etch adhesive systems.

Chlorhexidine has been recommended for use as a cavity disinfectant because of its antibacterial properties. Given these broad-spectrum antibacterial characteristics, it is frequently used in dentistry in toothpaste form, and as a mouthwash, gel or polish. Chlorhexidine is bacteriostatic at low concentrations and bactericidal at high concentrations (30, 32).

Türkün et al.(20) studied three different cavity disinfectants (Consepsis; Tubulicid red; Ora-5) in class V cavities, and reported that the application of chlorhexidine (Consepsis) containing disinfectant together with Clearfil SE Bond Prompt L-Pop had no negative impact on microleakage. But in a similar study on milk teeth, Tulunoğlu et al.(43) concluded that the application of chlorhexidine containing cavity disinfectant increases marginal leakage. In addition, Türkün et al.(20) reported that outcomes could be affected since adhesive resin bonding values for milk teeth are lower compared to those for permanent teeth.

Microleakage, one of the main problems in restorations, may lead to marginal discoloration and fractures, secondary decay, postoperative sensitivity and eventual pulpal inflammation (44).

In addition to dye staining, other techniques such as radioisotopes, air pressure, bacteria and SEM are also employed in the evaluation of microleakage (45,46). In our study, we selected the methylene blue penetration technique because it is easy, economical, quantitative and easily compared in the evaluation of various restoration techniques (47). Our results showed less microleakage in the groups not administered cavity disinfectant compared to those groups in which it was applied ($p<0.05$). However, even though greater microleakage was observed in the groups in which cavity disinfectant was applied, the difference was not statistically significant.

CONCLUSION:

The use of chlorhexidine gel as a cavity disinfectant in class II cavities has no negative effect on microleakage in self-etch adhesive systems. We recommend the use of cavity disinfectant to eliminate bacteria from the cavity surface in restorative procedures.

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