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Evaluation the effects of chlorhexidine and different liners on class II composite restoration microleakage

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ABSTRACT

Objectives: Microleakage of gingival margins is one of the causes of composite restorations failure. This study evaluated the effect of Chlorhexidine and different liners on class II composite restoration Microleakage.
Materials and Methods: This *in vitro* study was performed on 42 intact premolars. Class II preparations in mesial and distal of tooth were made with the gingival margins 1.0 mm apical to the CEJ. The teeth were assigned to 7 groups: Group 1: liner RXU (Rely X Unicem); Group 2: CHX+ RXU; Group3: liner PAN F2 (Panavia F2); Group 4: CHX+PAN F2; Group 5: liner RMGIC (resin-modified glass ionomer cement); Group 6: CHX+RMGIC; Group 7: as control received no liner and CHX. Cavities were restored with self-etch SE bond and Z-250 composite resin. The restorations were thermocycled and soaked in 2% fushin for 24 hours, then sectioned mesiodistally and viewed under a stereomicroscope for leakage. The data were analyzed using the Kruskal-Wallis and Mann-Whitney tests ($\alpha = 0.05$).
Results: All groups showed significant difference with control group ($p<0.05$). Between groups 1 and 5 that did not received CHX, group 3 showed higher microleakage than the others significantly ($p<0.05$). In CHX groups, groups 2 and 6 were significantly ($p<0.05$) different from each other but no significant difference was observed between them and group 4 ($p>0.05$).
Conclusions: The present study showed that using of liner can decrease microleakage, however, RXU and RMGIc reduced microleakage significantly compare to PAN F2. CHX did not reduced microleakage while increased RMGIC microleakage significantly.
KEYWORDS: Chlorhexidine, Dental Leakage, Dental Cavity Lining, Dental Restoration

1. INTRODUCTION

Posterior composite restorations have improved during the past decade and they are increasingly used as direct restorations in occlusal and occlusoproximal cavities. Esthetics, no mercury content, adaptation to round cavities, reinforcing the remaining tooth structure and preservation of teeth via minimal preparation are among the advantages of posterior composite restorations (Manhart, 2004).

The main drawback of composite restorations especially in Class II cavities is marginal microleakage and passage of bacteria, fluids and molecules from the tooth-restoration interface; this leads to hypersensitivity, secondary caries, pulp irritation and marginal discoloration (Ozel and Soyman, 2009). Microleakage is an important factor when assessing the clinical success of composite restorations especially when the restoration margin ends in the dentin (Powell, 1994).

Several techniques have been suggested to resolve this issue including the incremental application of composite resin, use of a transparent wedge and matrix band, use of autopolymerizing composite resin, use of soft-start curing method and the sandwich technique. In the sandwich technique, an interstitial layer is applied to reduce the marginal gap between the tooth and the restoration (Hagge, 2000). Using 3D finite element analysis, Ausieuo et al. showed that a thicker adhesive layer has a higher elastic relaxation effect due to stress transmission and thus, uniformly disperses the stress in the respective layer (Ausiello, 2002).

Use of resin modified glass ionomer cement (RMGIC) with the sandwich technique is suggested due to enamel and dentinbonding, its bacteriostatic properties, possessing a modulus of thermal expansion similar to that of enamel and dentin, slow polymerization reaction and low shrinkage. Due to improved mechanical and physical properties compared to conventional glass ionomers, RMGICs increase the quality and longevity of the restoration (Van Dijken, 1999).

Resin cements not only improve the retention of restorations but also have less solubility and lower microleakage in the oral cavity compared to other cements (Stawarczyk, 2011). These cements were first produced for cementation of crowns, inlays and onlays; however, some studies reported their potential use as a cavity liner for reduction of microleakage.

Self-adhesive resin cements have low technical sensitivity and working time. They are partially filled and expected to have mechanical properties superior to those of self-etch bonding agents. Their bonding mechanism is due to acidic monomers in their composition that penetrate into the demineralized collagen network. Tooth-cement-composite bond has a greater thickness than tooth-bonding-composite and thus, the former has a higher potential to resist shrinkage forces which per se leads to better stress distribution in the interstitial layer, dentin and composite (Al-Saleh, 2010).

Another problem related to microleakage is the presence of residual microorganisms after cavity preparation that can lead to secondary caries. Histological and bacteriological studies have shown that only a small part of a tooth remains free from bacteria after cavity preparation. Thus, use of an antibacterial agent is recommended following cavity preparation for disinfection and decreasing the risk of bacterial activity (Brännström, 1986).

Chlorhexidine has been recommended as an effective dentin disinfectant and has shown to be effective against S. mutans (Ersin, 2008). During the process of caries, dissolution of some dentin minerals exposes its organic matrix that can be destroyed by bacterial enzymes and protease enzymes of the host namely matrix metalloproteinases (MMPs) found in the saliva and the organic matrixof dentin. Metalloproteinases are responsible for destruction of the majority of extracellular matrix components such as different forms of intact and denatured collagens (Sulkala, 2007). The collagenolytic activity of dentin is strongly reduced by chlorhexidine; which is a potent MMPS inhibitor. Chlorhexidine minimizes or inhibits the self-destructive activity of exposed collagen fibersin incompletely formed hybrid layer and mayrelate to the longevity of the hybrid layer and bond strength (Carrilho, 2007). Moreover, chlorhexidine can be an adjunctive method for rehydration of mineralized dry dentin and provide the moisture required for the expansion of the collagen network. Furthermore, by preventing the destruction of exposed collagen fibrils and inhibiting MMPs, it can preserve bond strength (Hebling, 2005).

Some studies have shown that along with the use of self-etch bonding systems, CHX can reduce debonding over time. However, if applied along with a self-adhesive resin cement, CHX has no effect on bond durability (Shafiei and Memarpour, 2010; Zhou, 2009; Hiraishi, 2010). Due to controversial results regarding the applicationof CHX, we sought to assess the effect of CHX and different liners on microleakage of Class II composite restorations.

2. MATERIALS AND METHODS

This laboratory experimental study was conducted on 42 intact human premolar teeth without cracks, caries or previous restorations extracted for orthodontic purposes in the past 3 months. For disinfection, the teeth were immersed in 10% formalin (Kumar, 2005) for 48h. The soft tissue residues and calculi were removed and the teeth were stored in distilled water until the experiment. Two class II cavities were prepared in the mesial and distal parts of each tooth with gingival margins in dentin (1 mm below the CEJ) and 3mm buccolingual width, 6mm occlusogingival height and 1.5mm axial depth using #245 fissure bur (Diatech, Scissdertal, Switzerland) and a high speed handpiece along with water and air spray. Line angles were rounded with one motion of #1/2 tungsten carbide round bur (SS White, Great White Series, Lakewood, NJ, USA). No beveling was done in the cavity.

After the preparation of each 5 teeth a new bur was used. Cavity dimensions were evaluated again using a periodontal probe. If the prepared cavity was smaller than the required size, the cavity was extended. If the prepared cavity was larger than the required size, the specimen was excluded from the study. The teeth were randomly assigned to 7 groups of 6.

First, an Automatrix metal band (Kerr Hawe Adapt Super Cap Matrix, Kerr Hawe SA, Bioggio, Switzerland) was placed around the teeth and fixed from the outside with sticky wax (Sticky wax model cement-500G, Associated dental products Ltd, Swindon, SN5 4HT, UK) to increase the adaptation of the band. Next, the study groups were restored as follows:

Group 1: Rely X Unicem (3M ESPE, Seefeld, Germany) was applied by a microbrush in a thin layer to the gingival floor and axial wall. Self-etch primer dentin bonding agent (ClearfillSEbond, Kuraray, Japan) was then applied to all cavity walls according to the manufacturer's instructions and cured by Demi LED light-curing system (Kerr Corp., Orange, CA, USA) for 20s. A2 shade Z250 composite resin was then applied in 4 increments. The first increment was applied horizontally with 1 mm thickness and the next increments were applied obliquely 2 mm thick. Each layer was cured for 20s. The occlusal surface was then carved using a football-shaped multi-fluted carbide bur (Diatech, Scissdertal, Switzerland) along with water and air spray.

Group 2: Specimens were restored similar to group 1 with the exception that before the application of RXU cement, 2% CHX solution was applied to the cavity by a sterile microbrush for 20s and gently air dried for 5s. The cavity was then restored using composite resin as in group 1.

Group 3: Specimens were restored similar to group 1 with the exception that Panavia F2.0 resin cement with self-etch primer (Kuraray Dental Co., Okayama, Japan) was used instead of RXU according to the manufacturer's instructions.

Group 4: Specimens were restored similar to group 2 with the exception that Panavia F 2.0 was used instead of RXU according to the manufacturer's instructions.

Group 5: Specimens were restored similar to group 1 with the exception that RMGIC (Fuji II LC, GC Corporation) was used instead of RXU according to the manufacturer's instructions.

Group 6: Specimens were restored similar to group 2 with the exception that RMGIC was applied instead of RXU according to the manufacturer's instructions.
Group 7: This group was considered as the control group and no liner or CHX was used in the cavities. The cavities were restored with Clear fill SEbond dentin bonding agent and Z250 composite according to the manufacturer's instructions.

The restored teeth were stored in distilled water in an incubator (Thermo scientific Heraeas microbiological incubator, Lough borough, UK) at 37°C for 7 days. The teeth were then subjected to thermocycling with 1000 cycles at 5-55°C with 30s dwell time in each bath and transfer time of 15 s between baths. After thermocycling, two coats of varnish were applied to the coronal surface of teeth up to 1mm of the tooth-restoration margins; the apices were sealed by wax. Specimens were immersed in 2% fuchsin at 37°C for 24h and then rinsed under running water for 5 min. For evaluation of microleakage, the teeth were cut in half mesiodistally by a metal diamond disc (Axis Dental Company, Sybron Endo, USA) under abundant water spray. Specimens were then evaluated under a stereomicroscope (PZO, Warsaw, Poland) at 40X magnification. Dye penetration assessment of each slide was categorized into 5 scales:
0: No dye penetration
1: Dye penetrated into outer ½ of gingival floor
2: Dye penetrated into inner ½ of gingival floor
3: Dye passed the gingival floor and penetrated up to 2/3 of the axial wall
4: Dye completely penetrated into the axial wall and reached the DEJ
Data were analyzed using non-parametric Kruskal Wallis test and Mann-Whitney test. $P<0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

Frequency distribution of microleakage in the study groups is shown in Table 1. Kruskal Wallis test revealed significant differences between the study groups in terms of microleakage (P=0.000). Pairwise comparison of study groups with the control group by Mann Whitney test is demonstrated in Table 2. Based on the Kruskal Wallis test, significant differences were noted between groups without the application of CHX (groups 1, 3 and 5, P=0.003). Pairwise comparison of these groups by Mann Whitney test is shown in Table 4.

Kruskal Wallis test revealed significant differences between groups with CHX application that were restored with different cements (groups 2, 4 and 6, P=0.050). Table 5 shows pairwise comparison of these groups by the Mann Whitney test. The Mann Whitney test failed to find a significant difference between groups 1 and 2 with the lowest amount of microleakage (Tables 4 and 5).

Table.1. Frequency distribution of Microleakage in the study groups

Groups	Degree of micro leakage					
	0	1	2	3	4	Total
Group1 (RXU)	4(%33)	1(%8)	4(%33)	3(%25)	0(%0)	12(%100)
Group2 (RXU + CHX)	2(%17)	2(%17)	4(%33)	4(%33)	0(%0)	12(%100)
Group3 (Pan F2)	0(%0)	0(%0)	3(%25)	7(%58)	2(%17)	12(%100)
Group4 (Pan F2 + CHX)	0(%0)	1(%8)	3(%25)	8(%67)	0(%0)	12(%100)
Group5 (RMGIC)	0(%0)	5(%42)	4(%33)	3(%25)	0(%0)	12(%100)
Group6 (RMGIC+ CHX)	0(%0)	0(%0)	4(%33)	8(%67)	0(%0)	12(%100)
Group 7 (Control)	0(%0)	0(%0)	1(%8)	2(%17)	9(%75)	12(%100)

Table.2. Pair wise comparison of Microleakage between the experimental and control groups using the Mann Whitney test

Group	Experimental groups	P value
Control group	Group 1 (RXU)	p<0.001
	Group 2 (RXU + CHX)	0.011
	Group 3 (Pan F2)	0.011
	Group 4 (Pan F2 + CHX)	p<0.001
	Group 5 (RMGIC)	p<0.001
	Group 6 (RMGIC + CHX)	0.001

Table.3. Pair wise comparison of experimental groups with similar cements with and without the application of CHX (Mann Whitney test)

Groups	Groups	P value
Group 1 (RXU)	Group 2 (RXU + CHX)	0.538
Group 3 (Pan F2)	Group 4 (Pan F2 + CHX)	0.347
Group 5 (RMGIC)	Group 6 (RMGIC + CHX)	0.020

Table.4. Pair wise comparison of experimental groups with CHX application and different cements (Mann Whitney test)

Groups	Groups	P value
Group 2 (RXU + CHX)	Group 4(Pan F2 + CHX)	0.077
Group 2 (RXU + CHX)	Group 6 (RMGIC + CHX)	0.050
Group 4(Pan F2 + CHX)	Group 6 (RMGIC + CHX)	0.932

Table.5. Pair wise comparison of experimental groups without CHX application and different cements (Mann Whitney test)

Groups	Groups	P value
Group 1 (RXU)	Group 3 (Pan F2)	0.005
Group 1 (RXU)	Group 5 (RMGIC)	0.590
Group 3 (Pan F2)	Group 5 (RMGIC)	0.005

Microleakage of composite restorations occurs due to stress at the tooth-restoration interface; this is caused by polymerization shrinkage, thermal changes in the oral environment and mechanical fatigue due to masticatory forces. The most important reason is the polymerization shrinkage towards the stronger enamel-composite bond and light source. This microleakage causes post-operative tooth hypersensitivity and recurrent caries necessitating restoration replacement. Several techniques have been suggested to decrease this microleakage such as incremental and oblique application of composite in order to reduce shrinkage and stress at the tooth-restoration interface. By decreasing the volume of cured material and C-factor at the same time, microleakage is reduced (Agrawal, 2012). We also applied composite in oblique increments.

Several methods have been introduced for measuring microleakage; however, no gold standard exists in this respect (Agrawal, 2012). We used dye penetration technique in our study because this method does not require complex laboratory equipment and is non-destructive (Alani and Toh, 1997). Researchers suggest that the dye used in this technique should have particle sizes smaller or equal to the size of bacteria (about 2 microns). Thus, we used 2% fuchsin solution with particles smaller than the size of bacteria (Yavuz, 2006).

Open sandwich technique has some drawbacks as well namely the high solubility of liners especially RMGICs that are exposed to the oral environment, their higher abrasion and low physical properties compared to composite resins. Therefore, we used a closed sandwich technique in our study;because it is associated with higher clinical success rate (Kasraei, 2011).

Our study aimed to determine the microleakage of composite restorations using a liner and CHX. Mann Whitney test showed significantly lower microleakage of the experimental groups compared to the control group. Presence of a liner in the cavity floor and subsequently reduced composite bulk (Yazici, 2003) are among the reasons for reduced microleakage in these groups. Use of bonding agents and formation of a thin adhesive layer on the cavity floor (that has low potential for absorption of polymerization shrinkage stress) can be another reason for higher microleakage in the control group (Kasraei, 2011).

Pairwise comparison of experimental groups showed that Panavia F2 dual-cure resin cement had higher microleakage than RXU and RMGIC; this may be attributed to the polymerization shrinkage of this cement (Piwowarczyk, 2005). Moreover, RXU application does not need dentin preparation and its formulation contains multifunctional phosphoric acid methacrylates that can bond to tooth structure and form a complex compound with calcium ions or undergo physical reactions and form hydrogen or bi-polar bonds. Due to these reactions, RXU can bond to enamel and dentin and create an optimal seal at the tooth-cement interface and lower the microleakage (Piwowarczyk, 2005).

Our study showed that RMGIC also decreases the microleakage to a great extent compared to the control group. This finding is also in agreement with the results of Aboushala (Aboushala, 1995) and Swift (KIM and HIRANO, 1999) demonstrating that RMGIC has great potential for reducing microleakage when used as a liner beneath composite restorations. RMGIC has a slow setting-reaction and some flow that decreases the transmission of stress to the bond and subsequently, less microleakage. Mount (Mount, 1998) described an ion-rich layer that forms by the transposition of calcium and phosphate ions from the apatite by the carboxyl group of the glass ionomer liquid. By setting of GI, re-deposition of ions occurs at the tooth-cement interface.

Mitra (2009) reported a formless area resembling a hybrid layer and seemed to be a product of the reaction of RMGIC and dentin minerals. Moreover, RMGIC has a chemical bond formed by the ionic reaction of polyalkenoicacid carboxyl groups and hydroxy apatite calcium ions that have adhered to collagen fibers. This chemical bond is responsible for the excellent long-term durable bond, microleakage resistance and dentin sealability of these materials (Dursun, 2013). Breschi et al. showed that use of CHX regardless of its concentration decreased the gelatinolytic activity of dentin. Application of CHX also significantly decreased nanoleakage when using bonding agents or acid etchants even after 2 years (Komori, 2009).

In our study, self-etch primer Panavia F 2 resin cement, RXU self-adhesive cement and SEbond self-etch bonding were used and no significant difference was found between these two cements in terms of microleakage before and after application of CHX. Previous studies also demonstrated that CHX had no effect on bond strength and microleakage of direct restorations (de Castro, 2003; MuratTürküfi, 2004). Although in our study CHX had no significant effect on the two resin cements, reduction in microleakage was observed in Panavia F.2 following the application of CHX.

Partial penetration of resin was observed in self-etch adhesives due to the presence of exposed collagens in the hybrid layer that have been affected by the activation of MMPs (Sano, 1994). Panavia F 2.0 is a self-etch primer cement with a relatively high viscosity and acts as resin bonding. Thus, unsupported collagen fibers may still be present (Shafiei and Memarpour, 2010). This is probably why use of CHX in our study decreased microleakage although it could not completely prevent dye penetration.

In our study, CHX had no effect on the durability of RXU bond. This cement is applied to the dentin covered with smear layer without any adhesive application. Its reaction with dentin surface is very superficial. Thus, exposure of collagens and activation of MMPs may not be possible (Shafiei and Memarpour, 2010).

Applying CHX prior to the placement of RMGIC significantly increased microleakage. This phenomenon may be explained by the fact that CHX has strong cationic properties (Mohammadi and Abbott, 2009) and can react with anionic carboxyl groups in the RMGIC. Therefore, it prevents the formation of calcium carboxyl bonds and decreases the bonding ability of RMGIC to dentin (Dursun, 2013). Interference of CHX with the chemical bonding mechanism of RMGIC can decrease the resistance of the hybrid layer to hydrolytic destruction. Furthermore, CHX interferes with the second step of RMGIC setting by competing with aluminum ions for the carboxyl groups and inhibits the completion of RMGIC reaction (Dursun, 2013). These findings confirm the results of previous studies that stated CHX decreases the physical characteristics of RMGICs and their bond to tooth structure (Dursun, 2013; Sanders, 2002)

This *in-vitro* study was conducted on extracted teeth. In the clinical setting, other factors such as thermal, mechanical and chemical stressors and fatigue during function also affect the longevity and durability of the bond. Although we applied thermal stress to the specimens by thermo-cycling, future studies should be able to better simulate conditions of the oral environment.

4. CONCLUSIONS

Within the limitations of this study, the following conclusions can be drawn:

1. Application of a liner in CL II posterior composite restorations significantly decreased microleakage compared to the control group
2. Regardless of CHX application, RXU self-adhesive cement and RMGIC were equal in terms of reducing microleakage;both decreased microleakage significantly more than Panavia F 2.0 self-etch primer cement
3. Application of CHX did not cause a significant change in microleakage in the RXU and Panavia F 2.0 groups while the microleakage of RMGIC significantly increased.
4. Among all study groups, group 1 (RXU) showed the lowest microleakage; although its degree of microleakage was not significantly different from that of group 2 (RXU + CHX) and group 5 (RMGIC).

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