Journal of Dentomaxillofacial Radiology, Pathology and Surgery

Winter 2020, Volume 9, Number 1

Research Paper: Effect of saliva contamination and different decontamination procedures on micro leakage of composite restorations

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Citation: Darabi F, Tavangar SM , Tayefeh Davalloo R, Ebrahimi M, khanderu H. Effect of saliva contamination and different decontamination procedures on micro leakage of composite restorations. Journal of Dentomaxillofacial Radiology, Pathology and Surgery. 2020; 9(1):31-37. http://dx.doi.org/

http://3dj.gums.ac.ir

Article info:
Received: 2020/01/20
Accepted: 2020/03/28

Keywords:
Composite Resins, Dentin-Bonding Agent, Saliva

ABSTRACT

Introduction: A few studies have investigated the effect of saliva contamination of cured or uncured adhesive systems. The aim of this study was to evaluate the effect of salivary contamination and different decontamination methods on microleakage of composite restorations.

Materials and Methods: Class V cavities (2 mm wide, 1.5 mm deep, and 4 mm long) were prepared on buccal and lingual surfaces of 135 extracted human premolars. The specimens were randomly divided into 9 groups, 30 cavities in each. The materials used consisted of single bond (3M) and Z250 (3M). Except group 1 (Control), in Groups 2-5, uncured adhesive, and in groups 6-9 cured adhesive was contaminated with saliva (30 s). Decontaminating procedures were: blot-drying, rebonding (Groups 3 and 6), rinsing, air-drying, rebonding (Groups 4 and 7), rinsing, blot-drying, rebonding (Groups 8 and5). In groups 2and 9 no decontamination procedure was done. After restoring the cavities, thermo-cycling and dye penetration, they were sectioned buccolingually and analyzed by stereomicroscope. Data were analyzed by Kruskal-Wallis and Dunn tests ($P<0.05$).

Results: In occlusal margins; there were no significant differences in the microleakage between groups 3,4,5 with group 1($P>0.05$) but in gingival margin, there were significant differences in the microleakage between all of the groups with group1($P=0.0001$).

Conclusion: None of the methods in this study could reduce the micro leakage in the cavities with both enamel and dentin margins.

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Introduction

Composite resins are technique-sensitive, and achieving good isolation is very important. Unfortunately, it is not possible to use rubber dam in all clinical cases and, when using cotton rolls during the bonding procedures, some kind of contamination may happen.(1,2) Studies related to bonding efficacy of the saliva-contaminated bonding system or different decontamination procedures are controversial. Several studies have suggested that "total etching single bottle adhesive systems" are less sensitive to contamination with saliva than previous generation bonding agents.(2–7) Others have reported that saliva contamination of dentin resulted in a reduction of shear bond strength.(8–11) In addition, saliva contamination did not show the same effect in different stages of the bonding process.(5,6,12,13) Controversial data have been reported regarding the effect of saliva contamination on bond strength and microleakage of adhesives because it depends on the individual adhesive used and also few studies have investigated the effect of saliva contamination on microleakage of cured or uncured adhesive systems.(3,8–17) The aim of this study was to compare the effect of different treatments on microleakage of a single bottle adhesive contaminated with saliva before and after curing. The null hypothesis was that none of the decontamination procedures could recover microleakage after saliva contamination of cured or uncured adhesive.

Materials and Methods

In this interventional experimental study, 135 freshly extracted premolar teeth, stored in normal saline, were scraped of any residual tissue tags and cleaned with pumice. Standardized class V cavities (2 mm wide, 1.5 mm deep, and 4 mm long) were prepared on the buccal and lingual surfaces of the teeth with the incisal margins being at the enamel and the gingival margins being in the cementum/dentin. Using a random number table, the cavities were randomly divided into 9 groups of 30(n=30)

(Table 1).

Table 1: Materials used in this study

MATERIALS	MANUFACTURE	COMPOSITION
SINGLE BOND	3M ESPE	BIS-GMA,HEMA,DIMETHACRYLATES,ETHANOL, WATER, A NOVEL PHOTOINITIATOR SYSTEM, A METHACRYLATE FUNCTIONAL COPOLYMER OF POLY-ACRYLIC AND POLY-ITACONIC ACIDS.
37% PHOSPHOIC ACID GEL	DENFIL	H3PO4+ NATURAL POLYMERIC MATERIALS
FILTEK TM Z250	3M ESPE	BIS-GMA,UDMA,BIS-EMA, WITH SMALL AMOUNTS OF TEGDMA.

Group 1: No contamination = Control

All the enamel and dentin surfaces were etched (DenFilTM Etchant-37%) for 15 and 5s, respectively, and then washed vigorously with water. The excess water was removed using air until the enamel was chalky in appearance but the dentin was not desiccated. Application of Single Bond (3M ESPE) with a small saturated brush in two consecutive coats was followed by 5s of gentle air drying for removal of solvent and 20s light activation with a visible light curing unit, Optilux 500 (Demeton-Kerr, Orange, CA, USA) (800mW/cm2). Then, the composite Z250(3M ESPE) was inserted in two gingival and occlusal layers and each layer cured for 40s.

Group 2: The bonding procedure was carried out as in the control; however, the surface was contaminated with fresh saliva before light curing the adhesive and undisturbed for 20s. For contamination, 0.05cc of fresh human saliva was used by a Hamilton syringe for 30s. Then, without any decontamination procedure, adhesive was cured and composite was applied as in Group 1.

Group3: After saliva contamination of uncured adhesive, the saliva was blot dried

Darabi F, et al. Effect of saliva contamination and different decontamination procedures on micro leakage of composite restorations. Journal of Dentomaxillofacial Radiology, Pathology and Surgery. 2020; 9(1):31-37.150. http://dx.doi.org/

and adhesive application was repeated and light cured and then composite was inserted.

Group4: After saliva contamination of uncured adhesive, Saliva was rinsed with a water stream from an air–water syringe for 20s and then gently air-dried with an air–water syringe from 10 cm distance. The adhesive was reapplied and light cured and then the composite was inserted.

Group 5: As group 4, saliva was rinsed with a water for 20s but then blot dried using cotton pellets. The bonding procedure was repeated and light cured and then composite was applied.

Group6: In this group cured adhesive contaminated with saliva and decontamination procedure was similar to group 3.

Group7: Cured adhesive contaminated with saliva and decontamination procedure was similar to group 4.

Group 8: Cured adhesive contaminated with saliva and decontamination procedure was similar to group5.

Group 9: Cured adhesive contaminated with saliva but as group 2 without any decontamination procedure composite was inserted and cured.

After 24 hours, the restorations were finished to the cavosurface margins using a 12 fluted carbide-finishing bur (SS White burs Inc., Lakewood, NJ 08701) and soft-lex disks (3 M Dental Products. St Paul, S0144) before being thermo-cycled (5 to 55°C, dwell time: 30s, 500 cycles).

After thermo- cycling, the apices of the specimens were sealed with paraffin and all tooth surfaces were covered with two coats of nail varnish to approximately 1.0 mm from the restoration margin. The specimens were then immersed in 0.5% basic fuchsin dye at 37°C for 24 hours, rinsed cleaned from the nail varnish, embedded in epoxide resin and sectioned bucco- lingually at the center of the restorations with a diamond disc and low speed handpiece.

The amounts of microleakage were assessed for both of enamel and dentin margins by two calibrated examiners blinded to the test groups using an Olympus stereomicroscope SZX7 (Olympus corporation, Tokyo, Japan) (×30) and scored on a scale of 0 to 4 as follows:

0=No leakage

1=penetration less than or the length of occlusal/gingival wall

2=penetration greater than the length of occlusal/gingival wall

3=penetration up to axial wall

4=penetration along the axial wall

The data were analyzed using Kruskal-Wallis one-way ANOVA and multiple comparison (Dunn) tests.

Result

Score frequency and mean rank for microleakage are presented in table 2. The mean microleakage score in groups 2 and 9 (no decontamination) was highest as compared to those of the other groups. It is also noteworthy that the microleakage values in all groups were higher at dentin margins than enamel margins. Because of significant difference between the groups by Kruscal-Wallis test

($p<0.05$), Dunn test was used to pairwise comparison of the groups (table 3 and 4). At occlusal margin, there was no significant difference between control group (no contamination) with groups 3,4 and 5 (p value= 0.0895, 0.0895 and 0.1406 respectively) but with groups 6, 7 and 8 significant difference was found($p<0.05$).

At gingival margin, although there was significantly lower microleakage in groups 3,4 and 5 than 6, 7 and 8 ($p=0.0001$), but it was significantly higher than control group($p=0.0001$).

Table 2. Score frequency and mean rank for micro leakage at occlusal and gingival margins†

MARGIN	GROUP	SCORE FREQUENCY					MEAN RANK	P-VALUE
		0†	1	2	3	4		
OCCLUSAL	1	30*	0	0	0	0		<0.001
	2	9	20	1	0	0		
	3	24	5	1	0	0		
	4	23	7	0	0	0		
	5	24	6	0	0	0		
	6	12	17	0	1	0		
	7	16	13	0	1	0		
	8	17	12	0	1	0		
	9	10	20	0	0	0		
GINGIVAL	1	23	5	2	0	0		<0.001
	2	0	1	0	3	26		
	3	3	14	13	0	0		
	4	2	15	13	0	0		
	5	2	14	14	0	0		
	6	0	0	1	14	15		
	7	0	0	2	14	14		
	8	0	0	2	14	14		
	9	0	0	1	3	26		

* sample number

† Dye penetration scoring system; 0 = No microleakage, 1=penetration less than or length of occlusal/gingival wall, 2=penetration greater than length of occlusal/gingival wall, 3=penetration up to axial wall, 4=penetration along the axial wall

Table3: The results of pairwise comparisons at occlusal margin

GROUPS	1	2	3	4	5	6	7	8	9
1									
2	0.0001								
3	0.895	0.0003							
4	0.895	0.0003	1.0000						
5	0.1406	0.0001	0.8056	0.8056					
6	0.0001	0.6227	0.0015	0.0015	0.0007				
7	0.0001	0.1406	0.0275	0.0275	0.0144	0.3254			
8	0.0003	0.0859	0.0498	0.0498	0.0275	0.2192	0.8056		
9	0.0001	0.6227	0.0015	0.0015	0.0007	1.0000	0.3254	0.2192	

Table4: The results of pairwise comparisons at gingival margin

GROUPS	1	2	3	4	5	6	7	8	9
1									
2	0.0001								
3	0.0001	0.0001							
4	0.0001	0.0001	0.829						
5	0.0001	0.0001	0.6673	0.829					
6	0.0001	0.323	0.0001	0.0001	0.0001				
7	0.0001	0.104	0.0001	0.0001	0.0001	0.6673			
8	0.0001	0.104	0.0001	0.0001	0.0001	0.6673	1.0000		
9	0.0001	0.829	0.0001	0.0001	0.0001	0.187	0.0055	0.0055	

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Discussion

Salivary contamination of the operating field is a frequent problem in restorative procedures, especially when rubber dam isolation is difficult or impossible, e.g. in deep cervical lesions, incomplete tooth eruption or when an indirect aesthetic restoration is seated.

In the present study, natural saliva was chosen as the contaminant because artificial saliva may confound the results. In addition, many studies have accepted whole healthy human saliva as an acceptable contaminating medium.[4–12] Fresh whole human saliva was provided by a healthy female who was instructed to restrain from eating and drinking 1-2 h before saliva collection.

The decontamination methods used in this study were reapplication of dentin bonding after drying with cotton, washing and drying with cotton, washing and air-drying. Because of, these methods have shown acceptable bond strength in our previous study about saliva contamination of Single Bond (18).

There are a few articles regarding the effect of saliva contamination on microleakage and most of them are related to contamination after acid etching in total etch adhesive system or before applying of self-etch adhesive systems (17,19-21).

In our study, the presence of the highest microleakage in groups 2 and 9(saliva contamination without decontamination) indicates the effect of salivary contamination on increasing of microleakage. This finding is consistent with previous studies (11,16,17,19 -21).

In the present study, decontamination procedures could only reduce the microleakage values in the groups which contamination occurred before adhesive curing, but yet at gingival margin these values were significantly higher than those in control group. According to these results, the null hypothesis was partially rejected because the contaminated uncured adhesive\showed decrease in microleakage scores especially at enamel margin

In consistent with our findings, In the study of Fritz et al. (11), saliva contaminated cured adhesive, was resistant to decontamination procedures and the marginal gaps were higher than uncured groups. In study of Memarpour et al. (22), Saliva contamination after applying and light curing a self-etch adhesive (Scotch bond) had adverse effect on sealing property of fissure sealant and reapplication of adhesive didn't recover it.

Toodehzaeim and Rezaie(23) also reported that saliva contamination before or after primer application in two hydrophilic adhesive (Transbond and Assure) caused higher microleakage. Vieira et al. (24) used LV SEM (Low Vacuum Scanning Electron Microscopy) to detect saliva and the effect of decontamination procedures to

remove it. Their study showed that if contamination occurs after primer application (SE bond) or light curing the adhesive, decontamination using air drying or primer reapplication in the first case and drying or washing and drying in the second case is unable to remove saliva and prevent full adaptation.

Salivary substances such as glycoproteins, sugars, fatty acids, etc. easily absorb to the bonding layer and lower its surface energy (25). It seems that our decontaminating procedures may not eliminate the adsorbed residues of saliva contamination from cured adhesive (Single Bond), so reapplication of adhesive couldn't completely wet the surface and it created marginal gap. But removing the contaminants absorbed to uncured adhesive by drying or rinsing and drying seems to be more effective. However, SEM examination is needed to clarify these hypotheses.

Our previous study with the same materials showed that when saliva contamination occurs after light curing of Single Bond (3M), reapplying of adhesive followed by rinsing and air or blot drying is enough to restore shear bond strength (18). However, in the present study these decontamination procedures couldn't recover marginal seal in cured contaminated adhesive at both margins (enamel and dentin) and uncured adhesive at dentin margin. Although sealing property and bond strength of the restorations seem to be interdependent, both of them are also affected by independent variables and both are important in the qualitative evaluation of restorations.

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Conclusion

Under the circumstances of this study, it may be concluded that: none of the decontamination methods used in this study are effective in reducing the microleakage as much as the control group in the cavities where both dentin and enamel are present. Until further investigation, it is recommended to avoid contamination or resurfacing the contaminated surfaces.

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