AN INVITRO STUDY OF MICROLEAKAGE OF COMPOSITE RESINS WITH CHLORHEXIDINE CAVITY DISINFECTANT

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ABSTRACT

BACKGROUND

In routine dental practice, most of the patients want tooth colour restorations. Composite resin is a tooth colour restorative material. After removal of carious tooth structure, some microorganisms may remain in the cavity. To disinfect the cavity in this study chlorhexidine is used.

Chlorhexidine is a broad-spectrum biocide effective against Gram +ve bacteria, Gram -ve bacteria and fungi. It is both bacteriostatic and bactericidal. Chlorhexidine kills microorganism by disrupting cell membrane. The aim of this study was to evaluate the effect of Rexidin (a chlorhexidine gluconate-based mouth wash) application on composite restorations micro leakage, using two adhesive systems: 3M ESPE single bond universal Adhesive and Adhese.

MATERIALS AND METHODS

In this experimental study, class V cavities were prepared on labial surfaces of seventy-two extracted human incisors. The specimens were randomly divided into 6 groups (n=12): A1: Acid Eching (AE), 3M ESPE single bond universal adhesive; A2: AE, Rexidin, blot drying, 3M ESPE single bond universal adhesive; A3: AE, Rexidin, water rinsing, 3M ESPE single bond universal adhesive; B1: AE (only enamel margin), Adhese; B2: AE (only enamel margin), Rexidin, blot drying, Adhese; B3: AE (only enamel margin), Rexidin, water rinsing, Adhese. Afterwards, the cavities were restored with 3M Z100 composite resin restorative, thermo-cycled (5 to 50°C, dwell time: 30s, 1000 cycles,), immersed in 0.5% methylene blue for 24 hours and the dye penetration was evaluated and scored on a scale of 0 to 4 under stereomicroscope (×30). The data was analysed using Kruskal-Wallis and Multiple Comparison tests.

RESULTS

Statistically significant difference was found between groups B1 and B2 at both occlusal and gingival margins. ($P<0.05$).

CONCLUSION

Rinsing off the cavity disinfectant (Rexidin) before the bonding procedure does not affect the seal at the resin-tooth interface when using either of the adhesive systems; however, the sealing ability of Adhese seems to be inhibited by the remnants of the disinfecting agent.

KEYWORDS

Composite Resins; Dentin-Bonding Agents; Dental Leakage; Chlorhexidine, Acid Etching.

HOW TO CITE THIS ARTICLE: Venugopal L. An invitro study of microleakage of composite resins with chlorhexidine cavity disinfectant. J. Evid. Based Med. Healthc. 2019; 6(9), 658-662. DOI: 10.18410/jebmh/2019/137

BACKGROUND

Success in operative dentistry depends on total removal of the infected structure and achievement of a good seal;[1] however, the applied procedures for treating caries do not always eliminate all cariogenic microorganisms in residual tissues.[2-4] Although some authors believe that the number and pathogenicity of bacteria would be decreased once they are separated from the oral environment, the importance of the remaining bacteria in caries progression or pulpal involvement is emphasized by others.[5,6] Residual bacteria have been shown to proliferate from the smear layer even in the presence of a good seal from the oral cavity.[7] Other studies have shown that bacteria left in the prepared cavity could survive for a long time and this problem may be magnified by micro leakage of composite resin at margins not ending on enamel.[8,9] To solve this problem, the use of a disinfectant solution has been suggested.[10-14] Previous studies have depicted that a number of antibacterial solutions, such as chlorhexidine, sodium hypochlorite, fluoride based solutions and benzalkonium chloride, can be used as cavity disinfectants to eliminate residual bacteria from prepared cavities.[15,16] Some of the mentioned disinfectant solutions were found not to affect either the bond strength or the sealing ability of dentin bonding agents.[17-20] However, depending on the brand of materials and application methods, some of the solutions have shown an adverse effect on the issues mentioned.[21,22]

Financial or Other, Competing Interest: None.
Submission 16-02-2019, Peer Review 23-02-2019,
Acceptance 28-02-2019, Published 01-03-2019.
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DOI: 10.18410/jebmh/2019/137

Aims and Objectives

The purpose of this study was to evaluate the effect of Rexidin (2% chlorhexidine gluconate) on sealing abilities of two dentin bonding systems: 3M ESPE single bond universal Adhesive and Adhese.

MATERIALS AND METHODS

Seventy-two freshly extracted Human incisor 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 labial 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 teeth were randomly divided into six groups of twelve (Table 1).

Group A1 (Control Group): The cavity disinfectant was not used, and cavity surfaces were treated with 35% phosphoric acid, washed and blot dried. Then, the dentin bonding agent (3M ESPE single bond universal Adhesive) was rubbed on the surface for 10 seconds and light cured using an Optilux 500 curing unit (Demeton-Kerr, Orange, CA, USA) at 500 W/cm^{2}for 20 seconds.

Group A2: Rexidin, (Indoco) was applied after acid etching with a mini brush tip, left in contact for 20s and blot dried. Bonding procedures were performed as previously described (as in group A1).

Group A3: Rexidin was applied as in group A2 except that it was rinsed off for 15 s, air dried and then bonding procedures were performed as previously described.

Group B1: (Control Group): The cavity disinfectant was not used. First, the enamel margins were treated with 35% phosphoric acid, washed, and blot dried. Then, a self-etch 2-step dentin-bonding agent (Adhese, Vivadent Co., Schaan, Liechtenstein) was applied in the cavities according to the manufacturer's instructions and light cured for 20s.

Group B2: Enamel margins were treated with 35% Phosphoric Acid, washed, and blot dried. Rexidin was applied as in group A2 (without being rinsed) and bonding procedures were performed as in group B1.

Group B3: Rexidin was applied as in group B2 except that it was rinsed off for 15s, air dried and then bonding procedures were performed as previously described.

All the cavities were filled with two increments of 3M Z100 composite resin restorative material each cured for 40s. 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, 1000 cycles). After thermocycling, 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% methylene blue dye at 37°C for 24 hours, rinsed cleaned from the nail varnish, embedded in epoxide resin and sectioned labiolingually at the center of the restorations with a diamond disc and low speed hand piece.

Group	Acid Etching	Disinfectant	Dentin Bonding Agent
A1	Total-etc	No	3M ESPE Single Bond Universal Adhesive
A2	Total-etch	Rexidin 60s + Blot Drying	3M ESPE Single Bond Universal Adhesive
A3	Total-etch	Rexidin 60s + Water Rising	3M ESPE Single Bond Universal Adhesive
B1	Only Enamel Margin	No	Adhese
B2	Only Enamel Margin	Rexidin 60s + Blot Drying	Adhese
B3	Only Enamel Margin	Rexidin 60s + Water Rising	Adhese

Table 1. Treatment Groups

Margin	Group	Score Frequency					Mean Rank	p Value
		0	1	2	3	4		
Occlusal	A1	*10	1	2	0	0	17.21	0.797
	A2	8	3	1	0	0	20.04	
	A3	10	2	0	0	0	18.25	
Gingival	A1	6	2	3	1	0	16.38	0.349
	A2	2	2	5	2	0	21.92	
	A3	3	3	7	0	0	17.21	

Table 2. Score Frequency and Mean Rank or Micro Leakage in Group A at Occlusal and Gingival Margins

Margin	Group	Score Frequency					Mean Rank	p Value
		0	1	2	3	4		
Occlusal	B1	*12	0	0	0	0	15	0.026
	B2	7	3	2	0	0	22.67	
	B3	10	2	0	0	0	17.83	
Gingival	B1	8	3	1	0	0	14.29	0.057
	B2	2	7	2	1	0	23.36	
	B3	6	4	2	0	0	17.58	

Table 3. Score Frequency and Mean Rank or Micro Leakage in Group B at Occlusal and Gingival Margins

*Sample number, "– Dye penetration scoring system: 0 = No Micro leakage, 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.

The amounts of micro leakage were assessed for both of enamel and dentin margins by two calibrated examiners blinded to the test groups using a stereomicroscope (×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 analysed using Kruskal-Wallis one-way ANOVA and multiple comparison (Dunn) tests.

RESULTS

Kruskal-Wallis was carried out to compare the micro leakage mean ranks in A and B groups separately (Table 2, 3). There was no significant difference between mean ranks of the groups treated with 3M ESPE Single Bond Universal Adhesive (A1, A2 and A3) at both occlusal and gingival margins (P>0.05). However, there were such differences between the groups treated with Adhese (B1, B2 and B3) at the occlusal margin P=0.026) and also a slightly significant difference between those at the gingival margin (P=0.057). Dunn test revealed no significant difference between the specimens in groups B1 & B3, or B2 & B3 at either occlusal or gingival margins. However, a significant difference was found between the groups B1 and B2 at occlusal and gingival margins (P=0.008 and P=0.017 respectively).

DISCUSSION

According to the result of this study, using chlorhexidine with or without further rinsing prior to bonding did not adversely affect the sealing property of 3M ESPE Single Bond Universal Adhesive; however, doing so without rinsing prior to the application of Adhere significantly increased micro leakage scores. This may be indicative that there may have been some negative interactions between the remnants of the disinfectant and Adhese. It has also been stated that the use of a cavity disinfectant with composite resin appears to be material-specific regarding interactions with various dentin bonding systems.[18]

Only a few studies have revealed an increased amount of micro leakage when not rinsing chlorhexidine prior to dentin bonding agent application.[23,24] Tulunoglu et al[28] evaluated the effect of two disinfectants, one chlorhexidine based, and one alcohol based, as cavity washes prior to the application of two dentin bonding agents (Prime & Bond and Syntac). They found a remarkable increase in microleakage in deciduous teeth when the cavities were previously treated with a chlorhexidine based solution.[25] However, it is hard to compare their study with others due to some structural differences between primary teeth dentin and that in permanent teeth.[26]

According to the findings of Meiers and Kresin by scanning electron microscope (SEM), chlorhexidine-treated smear layers (without being rinsed off before bonding) were less affected by a self-etching primer and Tenure conditioner, indicating them to be more resistant to acidic materials. Nevertheless, the results of their study indicated that a 2% chlorhexidine cavity cleaner can be used as a cavity wash prior to the use of Syntac and Tenure without affecting their ability to prevent micro leakage. However, their findings cannot explain the greater amounts of microleakage in group B2 of our study at the occlusal/enamel margin because of using phosphoric prior to the application of chlorhexidine. Still, a surprising result from that study was the relative effectiveness of chlorhexidine (without using dentin bonding agent) in reducing the amount of microleakage. They explained this finding by a possible stabilizing effect exerted on the smear layer, turning it from a semi permeable, loosely bonded layer to a more impermeable, firmly bonded one.[18] In our study, Rexidin was used as a cavity disinfectant because in other studies chlorhexidine-based cavity disinfectant solutions displayed the most effective and the longest antibacterial activity, which will contribute to elimination of residual bacteria. Therefore, it is better not to rinse off the disinfectant if they would not have an adverse effect on the bonding process. Some clinicians prefer to apply the disinfectant before acid etching, but we think that the application sequence of the disinfectant depends on the generation of the bonding system. Total-etch adhesive systems operate by removing the smear layer and the subjacent dentin, so, it is more reasonable to disinfect the dentin after etching.

Although, self-etch dentin bonding systems affect the smear layer using a milder acidic monomeric primer with no rinse step necessitating the smear layer to be disinfected before using the acidic primer.[27]

The two dentin-bonding systems used in this study were 3M ESPE single bond universal Adhesive, a total-etch adhesive system, and Adhese, a two-step self-etching adhesive, with nearly the same formulation. They were chosen to examine how chlorhexidine would affect two different smear layer management techniques in different sequences of bonding according to their clinical use.

Theoretically, chlorhexidine could improve the sealing ability of the adhesives. Chlorhexidine has a strong positive ionic charge making capable of easily binding to phosphate groups. It has a strong affinity for tooth surfaces and this affinity is increased by acid-etching.

Chlorhexidine also increases the surface free energy of enamel and can as well have a similar effect on dentin.[24] Castro reported that a 2% chlorhexidine solution applied either before or after acid etching of the dentin does not interfere with the micro tensile bond strength of the composite resin to the dentin treated with either Prime Bond NT, Single Bond, or Clearfield SE Bond bonding systems. In addition, in the study of Soares et al,[25] the use of

chlorhexidine at concentrations of 0.12% and 2%, before, after or associated with acid etching did not significantly affect the micro tensile bond strength of Adper Single Bond 2 to dentin. There have also been studies advocating application of chlorhexidine on dentin after phosphoric acid etching has no adverse effect on bond strength of Single bond and even showing that after six months, the bond strength remains stable in chlorhexidine treated specimens while decreasing significantly in the control group.

SEM examination revealed that chlorhexidine solution deposits debris on the surface and within the tubules of the etched dentin while having no significant adverse effect on the shear bond strength of the composite to dentin using All-Bond2 adhesive system. It has also been depicted in the same study that dentin resin interfaces in these specimens were essentially the same as those in not treated with Chlorhexidine.[23] According to the results of our study, these deposits apparently interfered with the sealing ability of Adhese.

Cao et al,[27] believed that disinfectants decrease shear bond strength to dentin. However, the degree of the decrease is related to the brands of the adhesive and the disinfectant.

Meiers & Shook have also reported a remarkable decrease in the shear bond strength of a self-etching dentin bonding agent when the cavity was previously treated with chlorhexidine without being rinsed off.

Cavity prepared for restoration is never completely free from microorganisms/sterile, no matter whichever method of caries removal is followed, always a few microorganisms are left behind. Some authors say that once cavity is sealed by restoration, the microorganisms die out. But studies have shown that even for a period of 1 year the microorganisms which are left behind in cavity may be viable and are capable of causing secondary caries.

Chlorhexidine is a broad-spectrum biocide effective against Gram +ve bacteria, Gram -ve bacteria and fungi. It is both bacteriostatic and bactericidal. Chlorhexidine kills microorganism by disrupting cell membrane.

Murat and Ferit suggested that cavity disinfectants can improve the sealing ability of dentin bonding agents by remoistening the cavity prior to placing a dentin bonding agent that bonds to damp tooth structure. Schaeken et al have claimed that bound chlorhexidine molecules might serve as a co-surfactant on dentin surface.[28,29]

Adhese is a two component self-etch adhesive supplied in two bottles. Both the etchant and the primer are there in one bottle with the resin adhesive in the other one. The first bottle (primer) contains phosphoric acid acrylate, bis-acrylamide, water, initiators, and stabilizers and the second bottle (bonding agent) contains dimethacrylates, hydroxyethyl methacrylate, highly dispersed silicon dioxide, initiators, and stabilizers.

All these chemical compositions are employed in 3M ESPE single bond universal Adhesive as well except for bis-acrylamide and the solvent, which is ethanol. However, it seems that chemical residues left from chlorhexidine may contribute to a decrease in wettability of Adhese and a resultant decrease in its ability to impregnate the tooth surface. SEM examinations appear to be needed to clarify this hypothesis. Although in most cases the use of chlorhexidine has not exhibited an adverse effect on the sealing ability of dentin bonding agents, further investigations can be beneficial

CONCLUSION

Rexidin can be used as a cavity disinfectant with no further rinsing prior to the application of 3M ESPE Single Bond Universal Adhesive but it must be rinsed off before Adhese is applied in the cavity.

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