

Does Cavity Disinfectant Affect Sealing Ability of Universal Self-etch Adhesive?

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ABSTRACT

Aim: The purpose of this study was to evaluate 2% chlorhexidine disinfectant (CHX), chitosan, and octenidine dihydrochloride (as cavity disinfectants) on microleakage in cavities restored with universal self-etch adhesive.

Materials and methods: Eighty extracted human permanent premolars were selected. Class V cavities were prepared on the facial surface of each tooth. The teeth were then divided into four groups of 20 teeth each. For the control group after cavity preparation, no disinfectant was applied. The other 3 groups were treated with 0.1% chitosan, 2% CHX, and 0.1% octenidine dihydrochloride (OCT). All the groups were restored with universal adhesive followed by composite resin. The teeth were then immersed in 1% methylene blue dye and were sectioned buccolingually. Microleakage was checked under a stereomicroscope on both occlusal and gingival margins.

Result: Among all the groups chitosan-treated cavities showed the least microleakage. Chlorhexidine treated cavities showed less leakage as compared to control, OCT group at both the margins.

Conclusion: Chitosan as a cavity disinfectant improves the sealing ability of the self-etch adhesive. Furthermore, *in vivo* studies need to be conducted to examine the interaction and long-term effect of chitosan with the other self-etch adhesive systems.

Clinical significance: Chitosan a natural polysaccharide can be used as a cavity disinfectant as it improves the sealing ability of self-etch adhesive.

Keywords: Caries, Cavity disinfectant, Chitosan, Chlorhexidine, Octenidine dihydrochloride.

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INTRODUCTION

The success of restorative procedures depends on the effective removal of infected dentin, prior to the placement of the restorative material. The histological and bacteriological experiments have shown that viable organisms remain on the dentinal surface at the termination of routine cavity preparation, only a proportion of the teeth remain sterile after preparation.¹ Inadequate removal of the infected tooth structure can lead to microleakage, increased pulp sensitivity, pulpal inflammation, and secondary caries that may necessitate replacement of the restoration.² To reduce the potential for residual caries and sensitivity, after cavity preparation an antibacterial solution (cavity disinfectant) can be applied which can disinfect the dentin.³ The main focus of modern adhesive dentistry is increasing the marginal adaptation of restorations and their durability. The purpose of using adhesives in resin composite filling techniques is to establish a durable bond between the tooth structure and filling material. A problem in the use of a disinfectant with dentin bonding agents is the possibility of an adverse effect on the bond strength of the composite resins. Chlorhexidine having antibacterial properties against many bacteria is the most commonly used cavity disinfectant.⁴ Previous studies have proven that chlorhexidine preserves the bond strength by inhibiting matrix metalloproteinases.⁵ Chitosan, a natural polysaccharide obtained through partial deacetylation of chitin, is endowed with properties of biocompatibility, biodegradability, bio-adhesion, and toxicity. Prior studies have described the antimicrobial potential of chitin, chitosan, and their derivatives.⁶ Some studies have shown that chitosan has antibacterial and anti-plaque actions and anti-adhesive properties against *Streptococcus mutans* and other streptococci bacteria.^{7,8} Chitosan makes the dentin resistant to hydrolytic degradation, collagen degrading enzymes which can favor an increase in durability of adhesive restoration.⁹ Octenidine

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dihydrochloride (OCT) is a bispyridine antimicrobial compound that carries two cationic active centers per molecule and demonstrates broad-spectrum antimicrobial effects, covering both gram-positive and gram-negative bacteria, fungi, and several viral species.¹⁰ OCT is currently widely used in the medical field for skin burns and decontaminating mucous membranes and open wounds.

Need for the Study

There are no studies in the literature using OCT, chitosan as cavity disinfectant. The purpose of this study was to evaluate 2% chlorhexidine disinfectant (CHX), Chitosan, octenidine dihydrochloride (as cavity disinfectants) on microleakage in cavities restored with a single bottle adhesive.

MATERIALS AND METHODS

This *in vitro* study was conducted at Kalinga Institute of Dental Sciences, KIIT University, Bhubaneswar. Eighty extracted human permanent premolars that were free of caries, white spots, cracks, and restorations were selected. The teeth were cleared of debris and stored in a physiological saline solution containing 0.1% thymol at 37°C. Class V cavities were prepared on the facial surface of each tooth using a high-speed airtor handpiece, straight fissure diamond bur, and underwater spray coolant. Standardized preparations were obtained by making cavity preparations that had approximate dimensions of 3 mm occlusogingivally, 3 mm mesiodistally, and 1.5 mm depth parallel to the cemento-enamel junction (CEJ). The gingival half of the preparation was extended 0.1 mm below the CEJ gingivally. After each cavity preparation, a periodontal probe (Hu-Friedy, Dental Screening Probe) was used to check the accuracy of the dimension. Each preparation was rinsed with distilled water for 20 s and dried using filter paper. The teeth were then divided into four groups of 20 teeth each.

Group 1 (Control Group)

After cavity preparation, no disinfectant was applied. Self-etch bonding agent Adper™ Single Bond Universal (3M ESPE) was applied to the prepared enamel and dentin surface with a microbrush and left for 20 s, lightly air-dried for 5 s, and light-cured (Blue Phase, Ivoclar Vivadent) for 10 s. All the cavities were restored with one increment of composite restorative material Filtek Z350 XT (3M ESPE), light-cured for 20 s. The composite restorations were finished 24 h after completion of restorations using fine-grit finishing diamond burs (Diatech Dental AG), then polished with a series of sandpaper disks (Soflex, 3M ESPE).

Group 2 (Chitosan)

A 0.1% chitosan (KIIT Biotechnology, India) was applied on the cavity walls and floor using disposable micro applicator tips for 20 s, then dried with air for 5 s. Then, the procedure was repeated as in other group 1. A 0.1% chitosan solution was prepared by diluting 0.1 g of chitosan with 100 mL of 1% acetic acid and the mixture was stirred for 2 h using a magnetic stirrer.

Group 3 (Chlorhexidine)

A 2% chlorhexidine disinfectant (CHX) (Consepsis, Ultradent) was applied on the cavity walls and floor using disposable micro applicator tips for 20 s, then dried with air for 5 s. Then, the procedure was repeated as in group 1.

Group 4 (Octenidine Dihydrochloride)

A 0.1% octenidine dihydrochloride (OCT) (Tokyo Chemical Industry, Tokyo, Japan) was applied on the cavity walls and floor using disposable micro applicator tips for 20 s, then dried with air for 5 s. Then, the procedure was repeated as in the other 2 groups. A 0.1% OCT solution was prepared by mixing OCT with phenoxyethanol liquid.

Table 1: Distribution of microleakage scores at the occlusal and gingival margin (N = 80)

Group	Occlusal margin			
	Score	0	1	2
Control	0	2	10	8
Chitosan	5	10	3	2
Chlorhexidine	2	6	7	5
OCT	0	3	9	8
Group	Gingival margin			
	Score	0	1	2
Control	0	2	5	13
Chitosan	3	4	6	7
Chlorhexidine	1	3	4	12
OCT	0	1	6	13

The specimens were stored for 7 days in distilled water prior to leakage assessment. Thermocycling of 200 cycles was carried out at 5°C and 55°C, 15 s dwell time at low and high-temperature chamber (Thermocycling, HWB 332R, Thailand), respectively. After thermocycling, root apices of teeth were sealed with a layer of sticky wax and the entire tooth surfaces were covered with two layers of nail varnish except for 1 mm around the tooth restoration interface. The teeth were then immersed in 1% methylene blue dye for 24 h. After 24 h, teeth were thoroughly rinsed to remove excess dye, then invested in clear autopolymerizing resin. The teeth were sectioned with a diamond saw (NTI Diamond Disc, Kerr dental) buccolingually almost in the center of the restorations from facial to lingual surface. Microleakage was checked out under a stereomicroscope (Olympus Opto System, India) at 20× magnification on both occlusal and gingival margins. Microleakage scoring was conducted by a different operator who was not aware of the materials used to avoid bias in the study. The following scoring system was used as proposed by Munro et al.¹¹

0: No evidence of dye penetration.

1: Dye penetration along the interface to half of the cavity depth.

2: Penetration greater than half of the cavity depth, not including the axial wall.

3: Penetration involving axial wall.

RESULT

All the data were statistically analyzed by using the Friedman test. The results of dye penetration (leakage) were scored separately for occlusal and gingival margins (Table 1). Means and standard deviations values of all the groups are shown in Tables 2 and 3). Among all the groups chitosan-treated cavities showed the least

Table 2: Statistical comparison of microleakage at occlusal margins using Friedman test

Group	N	Occlusal				
		Mean	Std. deviation	Minimum	Maximum	Mean rank
Control	20	2.3000	0.65695	1.00	3.00	3.00
Chitosan	20	1.1000	0.91191	0.00	3.00	1.62
Chlorhexidine	20	1.7500	0.96655	0.00	3.00	2.40
OCT	20	2.2500	0.71635	1.00	3.00	2.98

Table 3: Statistical comparison of microleakage at apical margins using Friedman test

Gingival						
Descriptive statistics and ranks						
Group	N	Mean	Std. deviation	Minimum	Maximum	Mean rank
Control	20	2.5500	0.68633	1.00	3.00	2.72
Chitosan	20	1.8500	1.08942	0.00	3.00	1.95
Chlorhexidine	20	2.3500	0.93330	0.00	3.00	2.60
OCT	20	2.6000	0.59824	1.00	3.00	2.72

microleakage with mean rank (occlusal—1.62, gingival—1.95). No statistically significant differences were found in microleakage between the two groups (control, OCT) as shown in Tables 2 and 3. Chlorhexidine treated cavities showed less leakage as compared to control, OCT group at both the margins with mean rank (2.40, 2.60).

DISCUSSION

This study evaluated the microleakage of single component universal self-etch adhesive, at both the coronal and apical margins following the use of cavity disinfectants. Statistically significant differences were observed with respect to the scores of microleakage among the four groups at the occlusal and gingival margin. Microleakage was higher in the gingival margin compared to the occlusal margin, which was in agreement with the other studies.^{12–14} This was due to the presence of higher organic content, tubular configuration, fluid pressure, and lower surface energy of dentin (gingival margin) which made bonding relatively difficult than enamel (occlusal margin).¹⁴

Current dentin bonding systems are generally grouped into categories in terms of how they interact with the dentin smear layer. Self-etch system was used in this study as it is less technique sensitive by combining dentin conditioning and bonding agent in a single step eliminating the number of steps. The acidic component of primer of self-etch dissolves smear layer and incorporates it into the mixture as it demineralizes dentin and encapsulates collagen fibers and hydroxyapatite to form a hybrid zone. The risk of incomplete resin infiltration in total-etch system is eliminated by simultaneous infiltration of the exposed collagen fibril scaffold with resin up to the same depth of demineralization. As the moisture level of dentin is not a critical factor with these adhesives, the issue of wet bonding is not a concern with self-etch adhesive.¹⁵ Ultra mild self-etch adhesive (pH >1.5) Adper™ Single Bond Universal (3M ESPE) was used as it causes partial demineralization and allows chemical interaction between monomers and remaining hydroxyapatite crystal.

The cavities treated with chitosan, CHX revealed reduced microleakage at both coronal and apical margin as compared to OCT and control groups. Overall, chitosan revealed superior results (with mean rank occlusal—1.62, gingival—1.95) at both the margins. This was attributed to increase crosslinking between collagen fibers by chitosan which makes the dentin more resistant to hydrolytic degradation as well as collagen degrading enzymes.⁹ Chitosan increases the durability of adhesive restoration as it neutralizes matrix metalloproteinases. Fawzy et al. proposed that its use stabilized dentin collagen due to microfibrillar arrangements and produced dentin with superior mechanical properties.¹⁶ Another reason could be the acidic pH of chitosan being responsible for chelating Ca²⁺ ion in dentin resulted in depletion of inorganic material from smear layer.

Silva et al. proposed 0.2% chitosan used for 3 min removed smear layer efficiently.¹⁷ The chitosan polymer is formed by several dimers of chitin. The chitin dimer has nitrogen atoms with pairs of free electrons responsible for the ionic interaction between the metal and the chelating agent. The formation of complexes between chitosan and metal ions most probably is due to the mechanisms of adsorption, ion exchange, and chelation. As self-etch adhesives interact with the smear layer rather than eliminating it, their acidic potential may be buffered by the mineral content of a thick and dense smear layer.¹⁸ As chitosan removed smear layer efficiently, helps in better bonding and less microleakage.

The primary goal of dental restoration is to create a “perfect” seal, preventing leakage of contaminants contained in the oral environment. Microleakage has been defined as the “clinically undetectable” passage of bacteria, fluids, molecules, or ions between a cavity wall and the restorative material applied to it and can result from deterioration of the tooth-restoration interface, differences between thermal expansion coefficients of material-tooth tissue or polymerization shrinkage, causing staining, micro-gap formation, recurrent caries.¹⁹ Presence of bacteria in the smear layer after cavity preparation is the major cause of secondary caries. But it is still controversial as cavity disinfectant applied to reduce residual bacteria affect adhesion.

Chlorhexidine being a broad-spectrum antiseptic has been used for chemical plaque control for the past two decades and has also been used as a cavity disinfectant for many years.²⁰ CHX has an excellent rewetting capacity and a strong affinity to tooth structure which is thought to improve the bond strengths of the adhesive to dentin.²¹ Carrilho et al.²² proposed that 2% chlorhexidine application after acid etching preserves both the durability of the hybrid layer and bond strength *in vitro* of aged specimens. In this study, chlorhexidine-treated cavities showed less microleakage as compared to OCT, control (Tables 2 and 3). This is attributed to the inhibition of matrix metalloproteinases. Compared to chitosan, CHX treated cavities showed more microleakage. This may be due to CHX being water-soluble, dissociates it get absorbed into smear layer and make the dentin more resistant. Self-etch adhesive might not effectively demineralize the acid-resistant dentin, incorporate the CHX containing smear layer. This is in accordance with the study conducted by Singla et al.⁴ According to Meiers and Kresin, cavity disinfectants applied to dentin surfaces are resistant to acid conditioning.³ This acid-resistant layer might inhibit the ability of weak acidic primers to effectively demineralize the dentin and hydrophilic resin to impregnate dentin surface. This finding is in accordance with the results of Gurgan et al.,¹ Sharma et al.,² that using 2% chlorhexidine before or after etching the dentin without rinsing could adversely affect the shear bond strength of the dentin bonding agent resulting microleakage but in contrast to the study by Carrilho et al.²²

Octenidine dihydrochloride is active against gram-positive and gram-negative bacteria. OCT preparations are less expensive than chlorhexidine and no resistance has been observed.²³ OCT is unique due to its relative noncytotoxicity at the site of action and good antimicrobial activity. These characteristics make OCT attractive as a potential alternative antimicrobial agent to chlorhexidine.^{24,25} It is a dicationic surfactant; hence, it increases the surface free energy of dentin, thereby improving the wettability of the self-etch adhesive.²⁶ In contrast, our study showed more microleakage with OCT specimens as compared to chitosan, chlorhexidine which were statistically significant. This may be attributed to phenoxyethanol present in OCT preparation, which would have displaced more water from dentin that may be the reason for poor bonding leading to microleakage. Adper™ Single Bond Universal(3M ESPE) also contains ethanol which again removes excess water. As water is necessary to maintain collagen fibril expansion this loss of water results in poor bonding in the OCT group. Both OCT, control group exhibited almost similar values for microleakage.

In the present study, after composite restoration the specimens were subjected to thermocycling because it is a widely used method to simulate temperature changes that take place in the oral environment. Rosales-Leal investigated the sealing ability of etch and rinse and self-etching adhesives in class V cavities before and after thermocycling and observed that the thermocycling affected the marginal seal of self-etching adhesives.²⁷

For microleakage evaluation, dye penetration provides easy, fast, and commonly applied screening methods. However, the interpretation of the minimal differences in the results is difficult, and it reduces the sensitivity because of a qualitative feature of the method. The stereomicroscopic examination was chosen for this study as this provides a well-magnified two-dimensional view of the surface to be examined.²⁸ Advanced technologies like noninvasive cross-sectional imaging technique of optical coherence tomography and three-dimensional technique of microcomputed tomography should be carried out to assess the sealing ability of adhesive.

CONCLUSION

Within the limit of our knowledge comparison of these results with the literature becomes difficult since there are no studies related to chitosan, OCT as cavity disinfectant affecting sealing of self-etch adhesives. Reviewing the literature and correlating the inferences with the results of this study showed that application of chitosan as a cavity disinfectant improves the sealing ability of the self-etch adhesive. Furthermore, *in vivo* studies are needed to examine the interaction and long-term effect of chitosan with the other self-etch adhesive system.

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