

Comparative Evaluation of the Effect of Different Surface Contaminants on the Microleakage of two Different Generation Bonding Agents: An Invitro Study

Niket Kachhi^{1*} Milan Ughareja² Mihir Pandya³ Soham Patel⁴ Shashin Shah⁵ Akash Azad⁶

¹Senior Lecturer, Department of Conservative Dentistry and Endodontics, Goenka Research Institute of Dental Science, Gandhinagari, India.

²Reader, Department of Conservative Dentistry and Endodontics, Goenka Research Institute of Dental Science, Gandhinagari, India.

³Reader, Department of Conservative Dentistry and Endodontics, Goenka Research Institute of Dental Science, Gandhinagari, India.

⁴Reader, Department of Conservative Dentistry and Endodontics, Goenka Research Institute of Dental Science, Gandhinagari, India.

⁵Professor and Head, Department of Conservative Dentistry and Endodontics, Goenka Research Institute of Dental Science, Gandhinagari, India.

⁶Senior Lecturer, Department of Conservative Dentistry and Endodontics, Goenka Research Institute of Dental Science, Gandhinagari, India.

ABSTRACT

Aim: The aim of this in vitro study was to evaluate the microleakage of two different generation bonding agents in the presence of various surface contaminants.

Materials and methods: Class V cavities were prepared on 150 extracted human permanent molars. The samples were randomly divided into two main groups of 75 teeth each. Group I: Fifth generation bonding system (XP Bond). Group II: Seventh generation bonding system (Xeno V). Subgroups were formed according to exposure to different surface contaminants (saliva, blood, & haemostatic agent). Cavities were restored with nanocomposite (Ceram-X) and evaluated for microleakage. The scores were subjected to 't' test and ANOVA test.

Results: Both the bonding agents did not provide complete resistance to microleakage when there was no contamination. Microleakage was minimum in the no contamination subgroup and maximum with the haemostatic agent subgroup for both the groups. Xeno V was superior to XP Bond in bonding contaminated surfaces.

Conclusions: 7th generation bonding agent (Xeno V) showed less microleakage than 5th generation bonding agent (XP Bond) in presence of various surface contaminants used.

Keywords: Blood, Haemostatic agent, Microleakage, Saliva, Xeno V, XP Bond.

INTRODUCTION

There have been numerous advances in the generations of bonding agents, various techniques and its applications, since the last decade. According to Baren - "The way by which a composite resin adapts to the tooth substance depends more on the nature of the tooth surface than on the properties of the composite resin".^[1] In order to have successful adhesion between resin and tooth structure, it is necessary that the adhesive substrate should not be contaminated. Contamination at any step of the

bonding procedure can adversely impact the longevity of the restoration and decrease its clinical success.^[2] Contamination can lead to microleakage, which in turn leads to sensitivity, pulpal irritation, tooth discoloration, secondary caries and eventual loss of restoration and clinical failure.

MATERIALS AND METHODS

Sample selection

150 intact maxillary or mandibular extracted human permanent molar teeth were selected for the

Received: Mar. 11, 2018; Accepted: Apr. 18, 2018

*Correspondence Dr. Niket Kachhi.

Department of Conservative Dentistry and Endodontics, Goenka Research Institute of Dental Science, Gandhinagari, India.

Email: Not Disclosed

study. The teeth were stored in a solution of 0.1% (wt. /vol) thymol to prevent dehydration and bacterial growth.

Sample preparation

Class V cavities were made on the buccal and lingual surface of each tooth using a high speed hand piece with air/water spray and # 245 carbide bur. The size of the cavity was 2 mm wide, 1.5 mm deep and 6 mm long, paralleling the cemento-enamel junction (CEJ). The gingival half of the preparation was extended 0.5 mm below the CEJ. The enamel margins were beveled using a flame shaped carbide bur.

Division of samples into groups

The samples were randomly divided into two main groups of 75 teeth each:

- Group I: Cavities were treated with a fifth generation bonding system (XP Bond)
- Group II: Cavities were treated with a seventh generation bonding system (Xeno V).

The main groups were further divided into five subgroups according to exposure to different surface contaminants.

Subgroup A (control)

- Subgroup IA: Application of XP Bond according to the manufacturer's instructions.
- Subgroup IIA: Application of Xeno V according to the manufacturer's instructions.

Subgroup B (Saliva contamination)

Whole fresh unstimulated human saliva was collected from volunteers who had not eaten food or consumed any liquid for 30 minutes prior to the collection. The saliva was collected in a sterilized glass beaker for immediate use.

- Subgroup IB: After etching, fresh whole unstimulated human saliva was pooled over the bonding area with a microbrush and left undisturbed for 10 seconds. Rinsing was done for 10 seconds with air/ water spray. Excess was blot dried with tissue paper pellets. Priming and bonding was carried out, followed by curing.
- Subgroup IIB: After priming and bonding, fresh whole unstimulated human saliva was pooled over

the bonding area with a microbrush and left undisturbed for 10 seconds. Rinsing was done for 10 seconds with air/water spray. Excess was blot dried with tissue paper pellets. Repriming and bonding was carried out, which was followed by curing.

Subgroup C (Blood contamination)

At the time of the experiment, fresh whole blood was obtained from volunteers by pricking the spirit wiped forefinger with a lancet.

- Subgroup IC and IIC: Similar procedure as in the subgroup IB and IIB was done with blood as a surface contaminant.

Subgroup D (haemostatic agent)

- Subgroup IE and IIE: Haemostatic agent (Viscostat) was used as the surface contaminant in a similar manner as with saliva and blood.

Preparation of samples for dye penetration

All the prepared cavities were contaminated with respective contaminants and then restored with nano composite (Ceram-X, Dentsply) with each increment cured for 30 seconds with visible light curing unit. All the teeth were stored at 37 degree celcius for 24 hours in distilled water and then subjected to 3000 thermal cycles between water baths of $5^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and $55^{\circ}\text{C} \pm 2^{\circ}$ with dwell time of 30 seconds. Specimens were then stored in distilled water for 72 hours at room temperature. All the teeth to be subjected to dye-leakage tests were covered with two coats of nail varnish to within 1mm of the tooth-restoration margin, after the root apices were sealed with modelling wax. The specimens were immersed in methylene blue solution for 24 hours at 37 degree Celcius. After staining, teeth were rinsed to remove residual stain; and the radicular parts of the teeth were cut 4.5mm below the CEJ. Coronal parts were sectioned mesiodistally and then buccolingually in the approximate center of the restoration with a low speed diamond saw.

Microleakage was assessed for both occlusal (enamel) and gingival (dentin/cementum) margins using a stereomicroscope at original magnification X16.

The depth of the stain (dye leakage) was judged according to the following scale.

- 0 – No dye penetration
- 1 – Dye penetration upto one third of cavity wall.
- 2 – Dye penetration upto two third of cavity wall.
- 3 – Dye penetration onto the axial wall.

The graduations in microscope were converted into mm as follows :

No. of units = Reading in mm / magnification * zoom of eyepiece

To compare the linear dye penetration (microleakage), the specimens were subjected to “t-test” and ANOVA for inter group and intra group comparison.

RESULTS

Table 1: Intergroup comparison of linear dye penetration(microleakage) between 5th and 7th generation bonding agents on enamel margins with various surface contaminants.

Subgroup	Control group	Saliva contamination	Blood contamination	Heamostatic contamination
	IA/IIA (mm)	IB/IIB (mm)	IC/IIC (mm)	ID/IID (mm)
Mean (mm)				
XP Bond	0.200	0.453	0.560	0.666
Xeno V	0.126	0.213	0.213	0.253

Table 2: Intergroup comparison of linear dye penetration(microleakage) between 5th and 7th generation bonding agents on gingival margins with various surface contaminants.

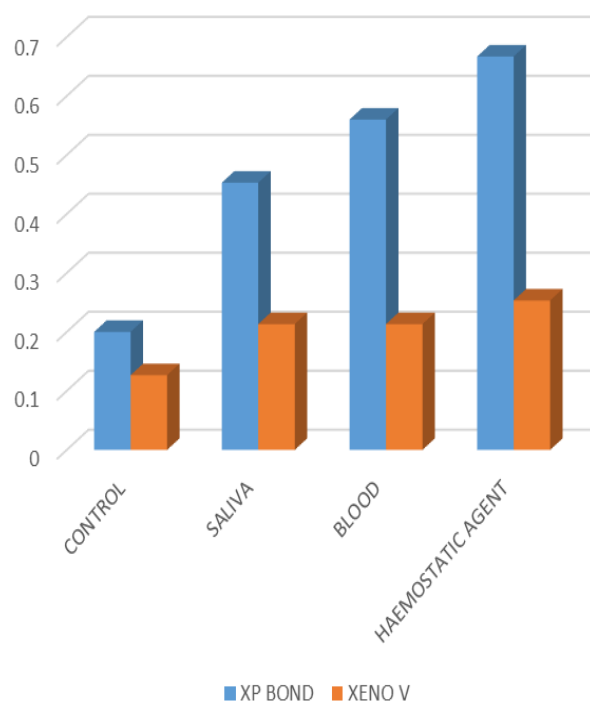
Subgroup	Control group	Saliva contamination	Blood contamination	Heamostatic contamination
	IA/IIA (mm)	IB/IIB (mm)	IC/IIC (mm)	ID/IID (mm)
Mean (mm)				
XP Bond	0.126	0.293	0.420	0.513
Xeno V	0.153	0.306	0.413	0.560

Table 1 shows intergroup comparison of linear Dye penetration (microleakage) between 5th & 7th generation bonding agents on enamel margin with various surface contaminants. Results showed that

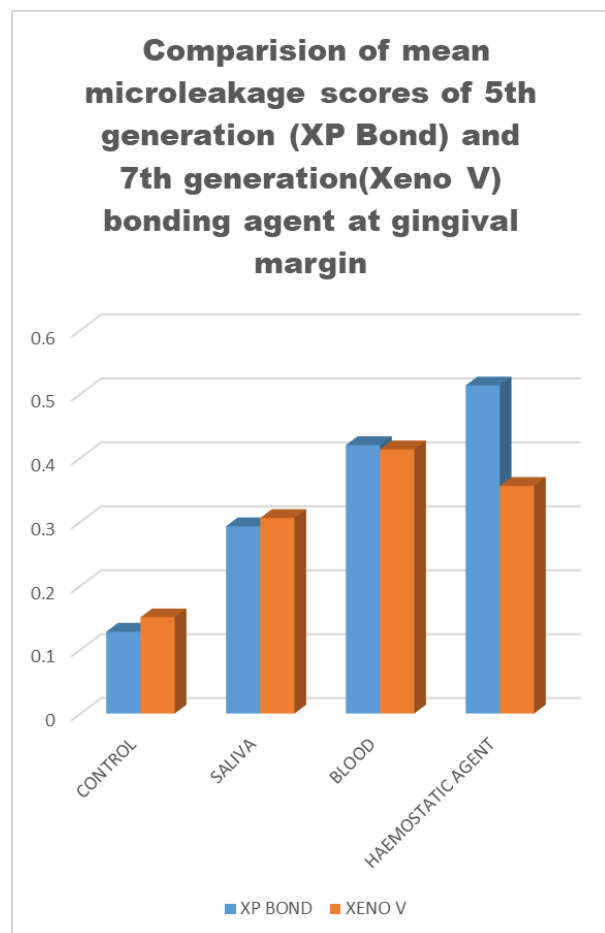
there was statistically more significant microleakage with 5th generation bonding agents (XP Bond) in all the groups at enamel margin compared to 7th generation bonding agents (Xeno V),with haemostatic agent showing maximum microleakage, while there was no statistical significant difference for blood and saliva group between XP Bond and Xeno V at enamel margin.

Table 2 shows intergroup comparison of linear Dye penetration (microleakage) between 5th & 7th generation bonding agents on gingival margin with various surface contaminants. Results showed that XP Bond showed more microleakage at gingival margin compared to Xeno V with haemostatic agent group showing maximum microleakage, followed blood, saliva, control group.

Comparison of mean microleakage score of 5th generation (XP Bond) & 7th generation (Xeno V) bonding agent at enamel margin



Graph 1: Comparison of mean microleakage score of 5th generation (XP Bond) & 7th generation (Xeno V) bonding agent at enamel margin.



Graph 2: Comparison of mean microleakage scores of 5th generation (XP Bond) and 7th generation (Xeno V) bonding agent at gingival margin.

DISCUSSION

It is widely believed that microleakage associated with resin composite restoration can be directly linked to marginal integrity.^[7] Microleakage can be defined as the passage of bacteria, fluid, molecules or ions between a cavity wall and the restorative material applied to it.^[4] The most significant factor in determining the resistance to postoperative sensitivity, recurrent caries, marginal staining and pulpal damage is the ability of the restorative material/adhesive to seal the restoration interface with the adjacent tooth substrate.^[6]

The physical properties of these resin restorative materials have improved over the years, especially in regard to their wear and fracture resistance and their use in stress-bearing posterior restorations. Despite their improvements, microleakage at the interface of the resin composite and tooth is still

considered to be a major problem. Thus, creating an effective seal between the resin composite and tooth structure remains critical factor in the longevity of resin composite restorations.^[8] Another characteristic of resin composite, which can affect their clinical success, is decreased bond strengths to dentin when compared to their bond strengths to enamel. Earlier microleakage studies have indicated that a resin adhesive bond to enamel margins exhibits less microleakage than those in non-enamel margins. There is evidence that the absence of enamel tooth structure in cavity margins located apical to the CEJ make them more difficult to seal.

Hence the present study was done to determine the microleakage in the 5th and 7th generation bonding agents in presence of various contaminants in Class V cavity preparations that were prepared on the facial and lingual surfaces of each tooth the gingival half of the preparation was extended 1mm below the CEJ.

The probable explanations for these results of Group IV (XENO V) include:

1. In Xeno V, tert-butanol is used as the solvent. The alcohol group in tert-butanol is shielded by the surrounding methyl groups of the tertiary group. Therefore tert-butanol is not able to chemically react by addition at the acrylate resins in the same way ethanol and isopropanol do. For this reason formulations containing tert-butanol are chemically more stable than those containing ethanol or iso-propanol.
2. Xeno V comprises newly developed and patented monomers. The novelty is due to the introduction of amide groups and "inverse" methacrylic ester functions instead of ester functions, resulting in more stability.
3. In addition to amide monomers, "inverse" functionalized monomers are used in Xeno V. These monomers are characterized by the attachment of the polymerizable methacrylate function to the acidic phosphoric acid ester function by a more hydrolysis-stable ether bond instead of an ester bond..
4. The type of solvent used in the adhesive systems: Instead of acetone, XENO V utilizes alcohol as the primer component solvent. It has

been proposed that acetone based adhesives require the use of wet bonding technique with a relatively small window of opportunity to achieve optimal hybridization. It also resulted in technique sensitivity which rendered it difficult to apply these adhesives properly in the complex cavity configurations. It was also difficult for the adhesives to infiltrate the demineralized collagen mesh fully and to remove all residual solvents, especially water, because of its low vapor pressure.

XP Bond showed more microleakage at the enamel margin than the gingival margin. The probable reason may be the pH of XP Bond. XP Bond has a pH of 2.5 which produces a shallow depth etch, and less abundant resin tag complex. On the contrary, Xeno V has pH <2, which produces better resin tag complex compared to XP Bond. Saliva contamination did not have significant effect on Xeno V; however, a very significant effect was seen on XP Bond at both the margins. This might be due to more technique sensitivity of XP Bond, and a difference in the chemical composition of two bonding agents. In the presence of salivary contamination, XP Bond showed a significantly greater microleakage at the enamel margin as compared to Xeno V. On the other hand, no significant difference was found between the two at the gingival margin. It has been shown that acid conditioned enamel surface readily absorbs salivary constituent, reducing the surface energy and rendering the surface less favourable for bonding.^[5] At enamel margin, the dried film of salivary protein inhibits penetration of the bonding agent into the hydroxyapatite. At the gingival margin, microleakage was due to protein absorbing properties of dentin.^[1] In this study, cavities were rinsed for 10 seconds with air/water spray after the exposure to surface contaminants. In contrast, various studies have concluded that re-etching after contamination is required to establish the shear bond that is comparable to the control group.

When the cavities were exposed to blood, Xeno V showed no significant microleakage at enamel margin; however, a significant microleakage was seen at the gingival margin when compared to control. XP Bond showed highly significant microleakage at both the margins when compared to control. The probable reason might be difference

in chemical composition of the two bonding agents. When comparing Xeno V with XP bond, XP bond showed a highly significant microleakage at the enamel margins, and not significant at the gingival margins. The probable reason could be some interaction between exposed collagen meshwork and blood protein components that could inhibit primer infiltration into dentin.

In Class V cavities with gingival margins below CEJ, thorough isolation is not always possible. Blood and gingival crevicular fluid due to inflammation of adjacent gingiva contaminate the prepared surfaces. The most common procedure used to control bleeding and decrease the flow of gingival fluid involves the use of topical haemostatic agent.^[4] Also, retraction cord impregnated with haemostatic agent can facilitate gingival tissue retraction.^[17] In the present study, when cavities were exposed to the contaminant haemostatic agent, (V-Feristat), Xeno V showed highly significant microleakage at gingival margin but no significant microleakage at enamel margin when compared to the control. XP Bond showed a highly significant microleakage at both the margins when compared to the control. When comparing Xeno V with XP Bond, highly significant microleakage was seen at enamel margin. The increased microleakage might be because it is a viscous gel composed of ferric sulphate. The viscous nature makes it harder to remove it from the dentin surface, and could potentially coagulate proteins present in the dentinal fluid.

The coagulated proteins and residues of ferric sulfate could in turn inhibit the infiltration of the bonding agent into the etched enamel surfaces and dentinal tubules. In a study, reduced bond strength most significantly when dried on the dentin surface. However with water rinsing and using cleansing agent (Concepsis), bond strength approached the control values. In another study, authors found that for cleansing the haemostatic agent contaminated surfaces by phosphoric acid, etching decreased the bond strength of self adhesive resin cement upto 18% to 21%. They recommended aluminium oxide particle abrasion for cleansing of surface contaminated by haemostatic agents.^[16]

Microleakage was not significant at enamel margin between all the surface contaminated subgroups of

Xeno V. This might be due to the cavosurface bevel at the enamel margin which exposes the rod ends. At the gingival margin for Xeno V, microleakage was significant when saliva group was compared with haemostatic agent subgroup. This might be due to the high viscosity of the haemostatic agent. Microleakage was not significant for all the subgroups of XP Bond at both the margins, except between saliva and haemostatic agent subgroup. This might be due to the composition of XP Bond and the viscous nature of the haemostatic agent.

The most probable reasons for the less microleakage at the enamel margins compared to that of the microleakage at the dentin/cementum margins are:

1. The hyper mineralization of the dentin surface and subsequent collapse of the collagen fibrillar network, Cavity configuration (C Factor), dentin tubule orientation to the cervical wall (CEJ), organic content of dentin substrate and movement of dentinal tubular fluids, incomplete alteration/removal of the smear layer by acidic primers for adequate demineralization and hybrid layer formation, inefficient infiltration/penetration of primer components into the demineralized collagen fibrillar network. Solvent carriers {water, alcohol, and acetone} in the adhesive agent reacting differently with varying degrees of surface moisture present in the tubules.^[11]
2. In class V cavities, located at approximately 1 mm from the CEJ, dentinal tubules are oriented parallel to the cervical wall. A classic hybrid layer formation is absent in the dentinal margins and this absence is an important cause of leakage.^[6]

CONCLUSION

1. Xeno V+ was superior to the XP Bond in bonding contaminated surfaces. In presence of various surface contaminants, Xeno V provided better marginal seal at enamel margin. XP Bond showed microleakage at both the margins
2. Gingival margins showed more microleakage compared to enamel margins.
3. Saliva contamination produced the minimum microleakage and haemostatic agent produced

the maximum microleakage for both the bonding agents.

Clinically, to achieve a successful bonding in Class V cavities, contamination should be avoided by thorough isolation and tissue retraction, prior to bonding. Adequate surface cleansing protocol should be taken into consideration if the contamination occurs before proceeding for the bonding procedure. However, additional in-vivo and in-vitro studies are desirable to further substantiate the findings of this study.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

REFERENCES

1. Baren IE. The adaptation of composite resin to tooth structure. The influence of cavity wall instrumentation upon the adaptation of composite resins and discussion. *Br Dent J* 1977;142:319-26.
2. John W, Evancusky JW, Meiers JC. Microleakage of compoglass-F and Dyract- AP compomers in class-V preparations after salivary contamination. *Pediatr Dent* 2000;22:39-42.
3. Idriss S, Abduljabbar T, Habib C, Omar R. Factors associated with microleakage in class II resin composite restorations. *Oper Dent* 2007; 32(1):60-6.
4. Al-Ehaideb AA, Mohammed H. Microleakage of one bottle dentin adhesives. *Oper Dent* 2001; 26: 172 -5.
5. Tay FR, Gwinnett AJ, Wei SH. Ultrastructure of resin dentin interface following reversible and irreversible rewetting. *Am J Dent* 1997;10:77-82.
6. Ben-Amar A. Microleakage of composite resin restoration. A status report for the American Journal of Dentistry. *Am J Dent* 1999;2:175-80.
7. Johnson ME, Burgess JO, Hermes CB, Buikema DJ. Saliva contamination of dentin bonding agents. *Oper Dent* 1994;19: 205-10.

8. Vinay S, Shivanna V. Comparative evaluation of microleakage of fifth, sixth, and seventh generation dentin bonding agents: An *in vitro* study. J Conserv Dent 2010;13:136-40.
9. Hilton TJ, Ferrencane JL. Cavity preparation factors and microleakage of Class II -composite restoration filled at intra oral temperatures. Am J Dent 1999;12:123-30.
10. Anusavice KJ. Phillips Science of Dental Materials. 11th ed. Missouri: Elsevier; 2004.
11. Kallenos TN, Al-Badawi E, White GE. An *in vitro* evaluation of microleakage in Class I preparation using 5th, 6th and 7th generation composite bonding agents. J Clin Pediatr Dent 2005;29:323-8.
12. Pashley DH, Nelson R, Kepler EE. The effect of plasma and salivary constituents on dentin permeability. J Dent Res 1982;61:978-81.
13. Hormati AA, Fuller JL, Denehy GE. Effects of contamination and mechanical disturbance on the quality of acid etched enamel. J Am Dent Assoc 1980;100:34-8.
14. Silverstone LM, Hicks MJ, Featherstone MJ. Oral fluid contamination of etched enamel surface: An SEM study. J Am Dent Assoc 1985;110:329-32.
15. Owens BM, Lim DY, Acheart KL. Effect of residual caries disclosing solution on microleakage of dental adhesive system. Quintessence Int (Berl) 2005;36:169-76.
16. Chaiyabutr Y, Kois JC. The effect of tooth preparation cleansing protocol on the bond strength of self-adhesive resin cement to dentin contaminated with haemostatic agent. Oper Dent 2011;36:18-26.
17. Liebenberg WH. Alternative gingival retraction techniques and isolation of cervical lesion. J Am Dent Assoc 1993;124:92-102.