

The Influence of Saliva on Gingival Marginal Microleakage of Class II Cavities when Using Universal Bonding Agent in Direct Composite Restoration

S Stoleriu¹, S Andrian, G Pancu, I Nica and G Iovan

¹Odontology, Periodontology and Fixed Prosthodontics Department, “Grigore T. Popa” University of Medicine and Pharmacy, Faculty of Dental Medicine, Iași, Romania

E-mail: stoleriu_simona@yahoo.com

Abstract. The aim of the study was to evaluate by microleakage assessment the effect of saliva contamination during bonding procedure when a universal bonding agent applied in two different strategies was used in direct restoration using composite resins. Standardized box-only Class II cavities were prepared on the mesial and distal surfaces of twenty extracted teeth. A hybrid composite resin (Gaenial Posterior, GC Corporation) and a universal bonding agent (G Premio Bond, GC Corporation) applied in two different strategies: etch-and-rinse and self-etch were used for restoration, with or without saliva contamination of the gingival margin of the cavity. Lower leakage was recorded in the cervical area apically than CEJ when universal bonding agent was applied in self etch strategy when compared to etch-and-rinse strategy. On enamel margins (1mm upper than CEJ) universal bonding agents applied in etch-and-rinse technique led to lower microleakage. Irrespective of etch-and-rinse or self-etch strategy of applying, the presence of saliva impaired the enamel or dentine leakage of universal bonding agents.

1. Introduction

The adhesion and retention of resin-based materials for direct restoration can be influenced by many clinical factors. In particular, moisture coming from saliva, blood, gingival fluid or sometimes from hand-piece oil [1] might affect the bonding to dental tooth structures and might lead to microleakage at the tooth restoration interface. Microleakage is responsible for biofilm accumulation, caries lesions onset adjacent to the restoration, postoperative sensitivity, marginal staining or even loose of the restoration [2].

It was stated that a good isolation of the tooth and an efficient prevention of contamination are mandatory to achieve a qualitative bonding. Cervical area of class V or class II cavities are more prone to contamination during clinical procedure of restoration using resin-based materials [3]. In addition, the thin gingival enamel margin or even the absence of enamel which exposes cementum/dentine substrate for bonding can influence the adhesion. In deep proximal box-shape cavities the quality of hybridization can also be negatively affected by the specific orientation of the dentinal tubules [4].

All dental adhesive systems are composed by three different components: etching agent, primer and bonding agent. Acid-etching might be applied as a separate clinical step (etch-and-rinse technique) or it might be provided by acidic functional monomers (self-etch technique). Previous studies reported a



significant decrease in bond strength between composite resins and enamel surface after saliva contamination of etched enamel [5] due to salivary proteins precipitation, which blocks the penetration of monomers in enamel pores and lead to a decreased mechanical adhesion [1]. The organic pellicle resulting from salivary proteins precipitation cannot be removed with water and it was demonstrated that re-etching the enamel for 10 seconds is needed to remove it [6].

In order to simplify the clinical procedure of bonding application, self-etch systems were introduced on the market. They have some advantages related to the elimination of rinsing and drying steps after acid etching, which might decrease the risk of saliva contamination and thus might allow a better adhesion [7-9]. In addition, some studies argued that self-etch adhesive systems are resistant to salivary contamination [2, 10].

The recent technology in dental adhesion is represented by the introduction of universal or multi-mode bonding agents. These materials consist in a mixture of all three components in the same bottle. According to the manufacturer indications, they can be applied either in etch-and-rinse and in self-etch procedure. There are studies that aimed to compare these bonding agents to previous generations of adhesive systems in terms of adhesion efficacy [11-13] or to evaluate their performance in direct composite repair [14, 15]. There is a lack of data in the literature regarding the efficacy of universal bonding agents when they are applied in the situation of moisture contamination.

The aim of the study was to evaluate by microleakage assessment the effect of saliva contamination during bonding procedure when a universal bonding agent applied in two different strategies was used in direct restoration using composite resins.

2. Experimental procedures

Twenty extracted molars having no cracks, caries lesions or restorations on the proximal surfaces were selected for this study. Two standardized box-only Class II cavities were prepared on the mesial and distal surfaces of each tooth having a width of 3 mm and a depth of 2 mm by a single operator. To prepare the cavities, a straight fissure diamond burs for every five cavities was used. The buccal and lingual walls of the preparations were parallel to each other and the line angles with the gingival wall were rounded. For a half of the cavities (n=20) the gingival margin was placed 2 mm below the cemento-enamel junction (CEJ) and for the other half (n=20) the gingival margin was placed approximately 1 mm upper than the CEJ. The cavities were restored using a hybrid composite resin (Gaenial Posterior, GC Corporation) and a universal bonding agent (G Premio Bond, GC Corporation) applied in two different strategies: etch-and-rinse and self-etch. Gaenial is classified as a hybrid composite with a combination of 2 types of pre-polymerized resin fillers of 16-17 µm (silica and strontium and lanthanoid fluoride), inorganic fillers larger than 100 nm (fluoroaluminosilicate) and inorganic fillers smaller than 100 nm (fumed silica). G-Premio Bond is a universal adhesive system containing a unique combination of three functional monomers (4-MET, MDP, MDTP). In etch-and-rinse strategy, 35% phosphoric acid etchant gel (3M-ESPE, St. Paul, MN, USA) was applied for 15 seconds on enamel and dentine using the tip of the syringe, then the acid was removed using the water spray from a dental unit and gently dry using the air spray. For bonding procedure, the application of the bonding system was made according to the producer instructions: using disposable applicator brushes, bonding agent was applied by rubbing the tooth surface for 20 seconds, gently air dried for 5 seconds using medium air pressure and then light cured for 20 seconds. In self-etch strategy the bonding procedure was the same as the previous one, except the acid etching step.

Human saliva was used to simulate the contamination of the cavities. Stimulated saliva was collected from one donor after chewing paraffin wax for five minutes. A quantity of 0,2 ml of saliva was applied on the gingival wall of a half of the prepared cavities using a brush applicator for 15 seconds, then the excess was spread using air spray from the dental unit. When the adhesive system was applied in etch and rinse strategy, contamination was simulated after etching and in self-etch strategy before bonding application.

In order to restore the cavities, a universal Tofflemire matrix retainer with matrix band was placed around the tooth and pressed with the fingers in the cervical area in order to prevent overhanging to the

gingival margin. Two horizontal layers of composite resin were used for each cavity restoration, each layer being placed using a dental spatula and a condenser, and being polymerized for 40 seconds using a LED light unit (LED B, Guilin Woodpucker Medical Instrument Co., Ltd, China) having the light intensity of $850\text{-}1000\text{mW/cm}^2$ and the wavelength of 420- 480 nm. Then the matrix was removed and the teeth were stored in distilled water for 1 week.

The distribution of the teeth in groups according to the strategy of bonding application was as follows:

Group 1 (n=5): cavity preparation with gingival margin lower than ECJ + bonding agent applied in etch and rinse strategy + restoration with composite resin

Group 2 (n=5): cavity preparation with gingival margin lower than ECJ + etching+ contamination with saliva +bonding application+ restoration with composite resin

Group 3 (n=5): cavity preparation with gingival margin lower than ECJ + bonding agent applied in self etch strategy + restoration with composite resin

Group 4 (n=5): cavity preparation with gingival margin lower than ECJ + contamination with saliva +bonding agent applied in self etch strategy + restoration with composite resin

Group 5 (n=5): cavity preparation with gingival margin upper than ECJ + bonding agent applied in etch and rinse + restoration with composite resin

Group 6 (n=5): cavity preparation with gingival margin upper than ECJ +etching+ contamination with saliva +bonding application + restoration with composite resin

Group 7 (n=5): cavity preparation with gingival margin upper than ECJ + bonding agent applied in self etch strategy + restoration with composite resin

Group 8 (n=5): cavity preparation with gingival margin upper than ECJ + contamination with saliva + bonding agent applied in self etch strategy + restoration with composite resin

The external surfaces of the teeth were coated with two layers of water resistant nail varnish, except the surface of the restoration and a area of 1 mm around the restoration, then the teeth were immersed in 2% methylene blue dye solution (pH=7) for 4 hours. After that the teeth were transversely sectioned using diamond discs (Komet Dental, Brasseler GmbH&Co, Germany), under cooling with water. The sections were examined using a optical microscope (Carl-Zeiss AXIO Imager A1m) coupled with a high resolution digital camera at 50X magnification. The dye penetration was assessed according to the following scores: 0- no dye penetration, 1- dye penetration on less than a half of the interface, 2- dye penetration on more than a half of the interface, but less than whole interface, 3- complete dye penetration of the interface, without involving the axial wall, 4. complete dye penetration of the interface, involving the axial wall. The sections were examined by two different evaluators, blinded to the method of bonding application. Examiners confronted the score given for each image and the final score resulted as a common decision of both examiners.

3. Results and discussions

The scores, the mean value of marginal leakage and standard deviation when the gingival margin was placed lower than CEJ are presented in Table 1.

Increased mean value of marginal leakage was recorded in group 2 when compared to group 1 and in group 4 when compared to group 3. In groups 3 and 4 the mean values of the leakage were lower when compared to groups 1 and 2.

Increased mean value of marginal leakage was recorded in group 6 when compared to group 5 and in group 8 when compared to group 7. In groups 7 and 8 the mean values of the leakage were higher when compared to groups 5 and 6. The values obtained in groups 5-8 were lower than that obtained in groups 1-5.

Three major factors were considered to be important in microleakage onset: polymerization shrinkage of composite resin, the biological substrate and the chemical composition of the bonding agent [16]. Also, thermal contraction, absorption of water, mechanical stress and dimensional changes in tooth structure

should affect the leakage [16]. Contamination with saliva of the operating field is a common clinical problem, especially when isolation using rubber dam is very hard to be achieved.

Table 1. Mean value of the scores for dye penetration \pm standard deviation (SD) when gingival margin was placed lower than CEJ.

	Group 1	Group 2	Group 3	Group 4
Scores	2	3	1	2
	3	3	2	1
	2	2	2	3
	3	2	2	3
	1	3	2	3
Mean score \pm SD	2.2 \pm 0.8	2.6 \pm 0.5	1.8 \pm 0.4	2.4 \pm 0.8

The scores, the mean value of marginal leakage and standard deviation when the gingival margin was placed upper than CEJ are presented in Table 2.

Table 2. Mean value of the scores for dye penetration \pm standard deviation (SD) when gingival margin was placed upper than CEJ.

	Group 5	Group 6	Group 7	Group 8
Scores	1	1	1	1
	1	0	0	2
	0	2	1	1
	1	1	1	1
	0	1	1	2
Mean score \pm SD	0.6 \pm 0.5	1 \pm 0.7	0.8 \pm 0.4	1.4 \pm 0.5

Different results were reported during the time regarding the effect of saliva contamination on the efficiency of adhesive systems. Some have reported that the saliva contamination had no adverse effect on the bonding efficiency of one-bottle adhesive systems [17-19]. Others have shown that the saliva contamination of the dentine surface produced a significant decrease in the bond strength [20-22].

In our study lower leakage was recorded in the cervical area apically than CEJ when universal bonding agent was applied in self etch strategy when compared to etch and rinse strategy. The same results were obtained by studies that aimed to compare the leakage of universal bonding agents applied in self etch technique and etch and rinse bonding agents [23, 24]. In etch and rinse strategy water is needed to keep the collagen fibril expansion for resin infiltration, but in the same time it has a bad effect on hybrid layer formation, decreases mechanical properties of the interface and lowers the durability of the bonded surfaces. Enzymatic degradation of exposed collagen fibrils and the hydrolysis of the adhesive polymer might appear as a result of uneven stress distribution in the hybridized zones [25, 26]. The popularity of self-etching adhesive systems increased over time due to „no-rinse” concept and lower postoperative sensitivity. By skipping the step of acid etching, the major concern of drying and wetting too much or too less is eliminated. Self etching agents etch only partially the dentine and some amounts of hydroxyapatite crystals are left in place around collagen fibers. Bonding stability relies on ionic bonds due to functional monomers (MDP) presence and affinity for hydroxyapatite [27, 28]. It seems that etch and rinse adhesive systems are not a good choice in terms of microleakage prevention when gingival margins are located in dentine [29]. On enamel margins (1mm upper than CEJ) universal bonding agents applied in etch and rinse technique led to lower microleakage.

Irrespective of etch and rinse or self etch strategy of applying, the presence of saliva impaired the enamel or dentine leakage of universal bonding agents. Some factors have been identified as possible causes for decrease the bond to contaminated dentine. The adsorption of salivary glycoprotein on tooth surface prevents the monomers penetrating to the dentine collagen network. Also, an increased contact angle might occur, which decreases the bond strength [20, 30]. The presence of saliva might dilute the primer, which determines a weak hybrid layer. Different results were reported in the literature regarding the effect of contamination on dentine adhesion. Some studies pointed that washing or washing and re-etching the dentine that has been contaminated led to no significant difference in bond strength [22]. On a contrary, Fritz concluded that re-etching is not necessary in case of contamination with saliva [20]. When saliva contamination happens after etching the dentine, blot drying of the dentine led to a bond strength equal to that obtained in case of uncontaminated surfaces [31]. Re-application of bonding agent after saliva contamination have been proposed to improve the bonding efficiency [32, 33].

4. Conclusions

Universal bonding agent applied in self etch strategy led to lower leakage in the cervical area located in dentine when compared to etch and rinse strategy. On enamel margins universal bonding agents applied in etch and rinse technique determined lower microleakage. Irrespective of etch and rinse or self etch strategy of applying, the presence of saliva impaired the enamel or dentine leakage of universal bonding agents.

5. References

- [1] Xie J, Powers J M and McGuckin R S 1993 *Dent. Mater.* **9** 295–9
- [2] Hitmi L, Attal J P and Degrange M 1999 *J. Adhes. Dent.* **1** 219–32
- [3] Mojon P, Kaltio R, Feduik D, Hawbolt E B and McEntee M I 1996 *Dent Mater* **12** 83–7
- [4] Kumar J S and Akshmi S J 2016 *J. Pharm. Sci. Res.* **8**(7) 627-631
- [5] Silverstone L M, Hicks M J and Featherstone M J. 1985 *J. Am. Dent. Assoc.* **110** 329–32
- [6] Hormati A A, Fuller J L and Deney J E 1980 *J. Am. Dent. Assoc.* **100** 34–8
- [7] Milia E, Lallai M B and Gracia- Godoy F 1999 *Am. J. Dent.* **12** 167–71
- [8] Brackett W W, Tay F R, Looney S W, Ito S, Haisch L D and Pashley D H 2008 *Oper. Dent.* **33** 89–95
- [9] Hürmüzlü F, Ozdemir A K, Hubbezoglu I, Coskun A and Siso S H 2007 *Quintessence Int.* **38** 206–12
- [10] EL- Kalla I H and Gracia- Godoy F 1997 *Am. J. Dent.* **10** 83–7
- [11] Kermanshah H and Khorsandian H 2017 *Dent. Res. J. (Isfahan)* **14**(4) 272–281
- [12] Gupta A, Tavane P, Gupta P K, Tejolatha B, Lakhani A A, Tiwari, Kashyap S and Garg G 2017 *J.Clin. Diagn. Res.* **11**(4) ZC53–ZC56
- [13] Somani R, Jaidka S and Arora S 2016 *Indian J. Dent. Res.* **27**(1) 86-90
- [14] Stoleriu Sandrian S, Nica I, Sandu A V, Pancu G, Murariu A and Iovan G 2017 *Materiale Plastice* **54**(3) 574-577
- [15] Andrian S, Pancu G, Topoliceanu C, Tofan N, Stoleriu S and Iovan G 2017 *Revista de Chimie (Bucharest)* **68**(8) 1874-1877
- [16] Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, Van Landuyt K, LambrechtsPand Vanherle G 2003 *Oper Dent.* **28** 213–324
- [17] Taskonak Band Sertgoz A 2002 *J. Oral Rehabil.* **29** 559–64
- [18] Yoo H M, Oh T Sand Pereira P N *Oper. Dent.* 2006 **31** 127–34
- [19] Yazici A R, Tuncer D, Dayangaç B, Ozgünaltay Gand Onen A 2007 *J. Adhes. Dent.* **9** 305–9
- [20] Fritz U B, Finger WJand Stean H 1998 *Quintessence Int.* **29** 567–72
- [21] Park Jand Lee K C 2004 *Oper Dent.* **29** 437–42
- [22] Ghavam Mand Pour Sh K 2004 *J Dent Tehran University of Medical Sciences* **1** 5–10

- [23] Van Landuyt K L, Peumans M, Fieuws S, De Munck J, Cardoso MV, Ermis RB, Lambrechts Pand Van Meerbeek B 2008 *J. Dent.* **36** 847–55
- [24] Ermis R B, Temel U B, Cellik E Uand Kam O. 2010 *Oper. Dent.* **35** 147–55
- [25] Pashley D H, Tay F R, Breschil B, Tjäderhane L, Carvalho R M, Carrilho Mand Tezvergil-Mutluay A 2011 *Dent Mater.* **27** 1–16
- [26] Jorge P 2007 *Dent. Clin. North Am.* **51** 333–35
- [27] Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda Rand De Stefano Dorigo E 2008 *Dent. Mater.* **24** 90–101
- [28] Armstrong S R, Jessop J L, Vargas M A, Zou Y, Qian F, Campbell J A and Pashley D H 2006 *J. Adhes. Dent.* **8** 151–60
- [29] Tuncer D, Çelik C, Çehrelı S Band Arhun N 2014 *J. Adhes. Sci. Technol.* 2014 **28** (13) 1288-97
- [30] Oztoprak M O, Isik F, Sayinsu K, Arun Tand Aydemir B 2007 *Am. J. Orthod. Dentofac. Orthop.* **131** 238–42
- [31] EL- Kalla I H and Gracia- Godoy F 1997 *Am. J. Dent.* **10** 83–7
- [32] Sattabanasuk V, Shimada Y and Tagami J 2006 *J. Adhes. Dent.* **8** 311–8
- [33] Eiriksson S O, Pereira P N, Swift E J Jr, Heymann H Oand Sigurdsson A 2004 *Dent. Materials* **20** 37–44