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Effects of Er:YAG Laser and 2% Chlorhexidine on Microleakage of a Self-Adhesive Agent (Bond Force) in Class V Composite Restorations

Shahin Kasraei[1], Ahmad Najafi-Abrandabadi[1], Hadi Kokabi-Arasteh[2], Mohadese Azarsina[3*]

[1]*Department of Restorative Dentistry, Dental School, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

[2]*Dental School, Hamadan University of Medical Sciences, Hamadan, Iran*

[3]*Department of Operative Dentistry, Dental School, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

ABSTRACT

Introduction: Investigating methods to decrease microleakage can improve the durability of composite resin restorations.
Aims: Evaluating the effect of surface treatments with Er:YAG (Erbium:Yttrium-Aluminum-Garnet) laser and chlorhexidine (CHX) prior to a self-etching adhesive on microleakage of class V composite restorations after a 6-months water storage.
Materials and Methods: Class-V cavities were prepared at the cement-enamel-junction of sound premolars (n=16). The study groups were as follows: control (C), 2% CHX application, Er:YAG laser treatment (2940 nm wavelength, 10 Hz pulse repetition rate, 0.5 W power) prior to bonding (Er), and after six months of water storage as follows: CW, CHXW, ErW. After application of Bond Force adhesive (Tokoyama Corp.), the cavities were restored with light-cured composite. The samples were stored in fuschin and marginal microleakage was assessed immediately and after six months of water storage. Data were analyzed by Kruskal-Wallis and Mann-Whitney-U tests ($p<0.05$).
Results and Discussion: CHX or laser had no significant effect on dentin and enamel marginal microleakage of restorations ($P>0.05$). Six months of water storage had no significant effect on microleakage at dentin and enamel margins ($P>0.05$). Microleakage at the enamel margins was lower than dentin margins before and after water storage ($P=0.001$).
Conclusion: Er:YAG laser or 2% CHX prior to the adhesive application had no effect on microleakage of class-V composite restorations.

Key words: Adhesives, Chlorhexidine, Composite resins, Dental leakage, Lasers

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Corresponding author: Mohadese Azarsina
e-mail✉: azarsina2012@yahoo.com
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INTRODUCTION

Recurrent caries is a common failure related to composite restorations. In composite restorations, the bonding agent forms a durable bond to enamel while the bond to dentin is challenging due to the complex composition of dentin [1]. A major shortcoming of currently used dental composites is polymerization shrinkage, which may result in gap formation at the tooth-restoration interface especially at the dentin margins [2]. Seepage of bacteria, fluids and ions through the tooth-restoration interface is referred to as microleakage [2], which increases the likelihood of marginal discoloration, recurrent caries, tooth hypersensitivity, pulp inflammation and necrosis [3]. To prevent this, advances have been made in the formulations of dentin bonding agents in the past two decades.

One-step self-etch adhesive systems simultaneously dissolve the inorganic components of dentin and diffuse into dentin collagen fibers by the activity of acidic monomers; this mechanism minimizes the likelihood of void formation in the hybrid layer [4]. However, microleakage at the dentin margins of restorations remains a clinical dilemma [3]. Some previous studies have recommended laser irradiation, application of chlorhexidine (CHX) and use of dentin bonding agents with antibacterial properties to decrease microleakage [5-9].

Er:YAG laser is used for cavity preparation, conditioning and pretreatment of enamel and dentin prior to the application of dentin bonding agents [5]. Erbium laser appears to be suitable for dentin-bonded restorations due to its capability to eliminate the smear layer and create an irregular or rough surface with open dentinal tubules with no thermal damage to dentin. Thus, some researchers believe that surface pretreatment with laser may increase the bond strength and durability of the restorations and decrease microleakage compared to the conventional methods [6,8,9].

Moreover, some studies have assessed the effects of chemical agents such as CHX on microleakage [5,8,10], and it has been reported that surface treatment with 2% CHX prior to placement of the restorative material decreases the incidence of recurrent caries and post-operative tooth hypersensitivity [5]. Aside from its antibacterial effects, CHX inhibits the activity of matrix metalloproteinases (MMPs) in dentin and enhances the quality of the hybrid layer [11]. Collagenase (MMP8) and gelatinase (MMP2-9) are present in dentin, and their destructive activity can be inhibited by protease inhibitors. Inhibiting MMPs and preventing the destruction of dentin collagen fibrils surrounded by penetrated resin can be a key to the success of dentin-bonded restorations. Improvement of methods to decrease marginal microleakage can enhance the clinical service of composite restorations.

This study aimed to assess the effect of cavity surface treatment with Er:YAG laser and 2% CHX prior to the application of Bond Force one-step self-etch bonding agent on marginal microleakage of class V composite restorations immediately and after six months of water storage. The null hypothesis was that Er:YAG and 2% CHX surface treatments prior to the application of Bond Force would not decrease microleakage at the enamel and dentin margins.

MATERIALS AND METHODS

This *in vitro* experimental study was conducted on 48 sound human premolars without any caries, cracks, cervical abrasion, restoration or congenital anomalies. The teeth were randomly divided into six groups. Class V cavities with 2 mm depth, 3 mm of mesiodistal dimension and 2 mm of occlusogingival width, measured with a Williams periodontal probe, were created on the buccal and lingual tooth surfaces at the CEJ in such a way that 1 mm of the cavity width was above the CEJ and within the enamel and 1 mm of cavity width was below the CEJ within the cementum or dentin (N=16). Enamel margin of the occlusal wall of the cavity was beveled (45° with 0.5 mm width). The cavities were prepared with high-speed hand piece and cylindrical diamond bur with 1 mm diameter under water and air coolant. The burs were changed after preparing eight cavities. Composite restorations were performed as follows:

In the control (C) group, no surface treatment was done on the tooth surface before bonding. After cavity preparation, the cavity was first dried and isolated and then Bond Force one-step self-etch adhesive agent (Tokuyama Dental Corporation, Tokyo, Japan) was applied on the cavity walls with a micro-brush for 20 seconds according to the manufacturer's instructions. After that, it was gently air dried for 5 seconds. Excess bonding agent was sprayed off. Light curing was performed using an LED light-curing unit (Demi Plus, Kerr Corporation, Middleton, USA).

In the CHX group, 2% chlorhexidine (Consepsis, Ultradent Products, Jordan, USA) was applied on the prepared cavity walls for 20 seconds by a swab. Excess material was removed by 10 seconds of air spray. Bond Force bonding agent was applied according to the manufacturer's instructions.

In the Er:YAG laser group (Er), the enamel and dentin surfaces were irradiated with Er:YAG laser (Fidelis3 Plus, Fotona, Slovenia) at exposure settings of 2940 nm wavelength, 50 µs pulse duration, 10 Hz pulse repetition rate, 50 mJ/pulse energy and 0.5 W power. Surface treatment was performed using R14 hand piece with a sapphire tip (13 mm length and 0.8 mm diameter) manually from 0.5 mm distance at a relatively perpendicular angle. The surface was horizontally scanned by sweeping motion at a speed of 1 mm/second. After surface treatment with laser, Bond Force was applied as described for the control group.

After bonding procedures, cavities in each group were restored with Z250 composite (3M ESPE, St. Paul, MN, USA) *via* incremental application of composite in three oblique layers. The gingival area of the cavity was filled first; then the occlusal layer was applied. The final increment completely filled the cavity. Each layer was light cured for 40 seconds. Samples were stored in distilled water at room temperature for 24 hours and were then subjected to 1000 thermal cycles. Each thermal cycle lasted for 90 seconds and included 30 seconds at 5 ± 1°C, 30 seconds at room temperature (24°C) and 30 seconds at 55°C ± 1°C.

In the CW, CHXW and ErW groups, methods of surface treatment were similar to those in groups C, CHX and Er, respectively. But, the only difference was that after thermocycling, specimens were stored in a bain-marie bath in distilled water at 37°C for six months.

For assessment of microleakage in each group, the apices were sealed with sticky wax and the external surfaces of the teeth were coated with two layers of nail varnish with one millimeter margin around the restoration. After drying, samples were immersed in 0.5% fuchsin for 48 hours, washed and dried. Samples in each group were sectioned by a diamond disc under water coolant in bucco-lingual direction longitudinally at the center. A single operator scored microleakage by twice observation of specimens under a stereomicroscope (SZ40, Olympus, Tokyo, Japan) at 40X magnification. Microleakage (*via* assessment of dye penetration depth) was scored as follows: Grade 0: No dye penetration/leakage; Grade 1: Dye penetration to half the lateral cavity wall; Grade 2: Dye penetration exceeding half the lateral cavity wall but not penetrating into the axial wall; Grade 3: Dye penetration to the entire cavity depth including the axial wall. During repeated observations, the higher value was chosen as the marginal microleakage. Data were statistically analyzed using SPSS version 16 (Microsoft, IL, USA), the Kruskal Wallis and the Mann Whitney U tests. Significance level was considered at α=0.05.

RESULTS

Table 1 indicates the marginal microleakage of composite restorations in different groups. The Kruskal Wallis test showed that surface treatment of cavities with CHX and laser had no statistically significant effect on the microleakage of Bond Force one-step self-etch bonding agent (P=0.55).

Comparison of microleakage in different groups revealed no significant difference immediately after thermocycling among C, CHX and Er:YAG groups in the enamel or the dentin margins of composite restorations (P=0.604 and P=0.738, respectively) (Table 2). Comparison of microleakage in the Er:YAG and CHX groups with the control group after 1000 times of thermocycling and six months of water storage revealed no significant difference in microleakage at the enamel or dentin margins of class V composite restorations (P=0.785 and P=0.458, respectively) (Table 2). Therefore, the complementary analyses for pairwise comparisons were not necessary.

Table 1: Marginal microleakage of composite restorations in study groups

Group	Storage Time	Margin	Grade 0 Number (%)	Grade 1 Number (%)	Grade 2 Number (%)	Grade 3 Number (%)
Control	No Storage	Enamel	8 (50)	5 (31.25)	2 (12.5)	1 (6.25)
		Dentin	3 (18.75)	4 (25)	3 (18.75)	6 (37.5)
	6 months	Enamel	4 (25)	7 (43.75)	4 (25)	1 (6.25)
		Dentin	2 (12.5)	3 (18.75)	3 (18.75)	8 (50)
CHX 2%	No Storage	Enamel	9 (56.25)	5 (31.25)	1 (6.25)	1 (6.25)
		Dentin	5 (31.25)	3 (18.75)	2 (12.5)	6 (37.5)
	6 months	Enamel	4 (25)	9 (56.25)	2 (12.5)	1 (6.25)
		Dentin	2 (12.5)	4 (25)	7 (43.75)	3 (18.75)
Er:YAG	No Storage	Enamel	11 (67.75)	3 (18.75)	1 (6.25)	1 (6.25)
		Dentin	4 (25)	4 (25)	5 (31.25)	3 (18.75)
	6 months	Enamel	2 (12.5)	10 (62.5)	4 (25)	0 (0)
		Dentin	1 (6.25)	4 (25)	7 (43.75)	4 (25)

Table 2: Comparison of microleakage at the enamel and dentin margins of Class V composite restorations

Restoration Margin	Storage Time	Groups	Mean	P-value*
Enamel[a]	No Storage	C	26.56	0.604
		CHX	24.75	
		Er	22.19	
	6 months	CW	25	0.785
		CHXW	22.75	
		ErW	25.75	
Dentin[a]	No Storage	C	26.44	0.738
		CHX	24.34	
		Er	22.72	
	6 months	CW	27.62	0.458
		CHXW	21.75	
		ErW	24.12	

*Krauskal Wallis Test; n=16 in each study group; similar letters in the table indicates the significant difference between groups with Mann-Whitney test.

Analysis of the data using the Mann Whitney U test revealed that irrespective of the type of surface treatment or long-term water storage, microleakage at the enamel margins was significantly lower than at the dentin margins of composite restorations (P=0.001). Moreover, long-term water storage (six months) had no significant effect on microleakage at the dentin and enamel margins of groups (when group C was compared with CW, CHX was compared with CHXW and Er was compared with ErW) (P>0.05).

DISCUSSION

This study aimed to assess the effect of cavity surface treatment with Er:YAG laser and 2% CHX prior to the application of Bond Force one-step self-etch bonding agent on marginal microleakage of class V composite restorations immediately and after six months of water storage. Bonding ability of composite resin minimizes the microleakage at the tooth-restoration interface and is an important factor predicting the clinical success of restorations. Dentin is a dynamic substrate containing considerable amounts of water and organic materials, which compromise the bonding strength with current adhesive systems. In the present study, microleakage at the dentin margins was significantly higher than the enamel margins (P=0.001). Thus, as confirmed in previous studies, microleakage at the dentin/cementum margins of class V composite restorations is greater than that at the enamel margins [5,12]. The force generated by the polymerization shrinkage of composite resin can compromise the bond strength and cause gap at the tooth-restoration interface, especially at the dentin margins.

The results of the current study showed that application of 2% CHX and laser irradiation prior to one-step self-etch bonding agent decreased microleakage. However, considering all the margins (enamel and dentin), this reduction was not significant in CHX and Er groups compared to the control group (P=0.55). Therefore, the null hypothesis of the study was accepted. Some previous studies also confirmed no significant effect of CHX on microleakage of composite restorations [2,5,10,13,14].

Gendron et al. claimed that CHX has optimal anti-proteolytic properties and can decrease long-term microleakage at the margins of composite restorations [15]. Two different mechanisms have been suggested for inhibition of MMPs by CHX. First is the chelating mechanism for inhibition of MMP2 and MMP9 and second is that CHX may interact with essential sulfhydryl or cysteine groups in the active areas of MMP8 [15]. Some previous studies have reported improved durability of bond and hybrid layer by the use of CHX [16-18]. However, some others have reported that application of CHX before the application of bonding agent increases microleakage. This may be due to the negative interaction of CHX and dentin bonding agents [8,19].

Arslan et al. reported that CHX not only did not reduce the microleakage at the enamel or dentin margins when total-etch and self-etch bonding agents were used; even it increased the microleakage at the enamel margin when self-etch bonding agent was used [8]. This was in accordance with the results of current study.

The existing controversy in the results of previous studies may be explained by the effect of concentration of CHX on its mechanism of action in the process of bonding to tooth structure. In most previous studies as well as the current investigation, high concentration (2%) of CHX was used, which may result in protein denaturation that adversely affects resin penetration into the hybrid layer. It seems that the CHX concentrations that have been used so far (to inhibit MMPs and their destructive effect on exposed collagen fibers) have been higher than the required value. Future studies are recommended to find the ideal concentration of CHX to increase the durability of bond to tooth structure.

The current study also showed that pretreatment of dentin and enamel surfaces with Er:YAG laser prior to the application of a one-step self-etch bonding agent had no significant effect on marginal microleakage of restorations. Some previous studies reported similar results as well, which is in agreement with our findings [5,20-22].

Arslan et al. reported that surface preparation with Er:YAG laser before the application of self-etch bonding agent decreased the microleakage at the enamel margins of class V composite restorations [8]. In contrast to the above-mentioned findings, Arslan et al. in another study in 2011 reported that in use of etch and rinse adhesive, no significant difference was noted in microleakage of class V composite restorations subjected to surface treatment with erbium laser and 2% CHX [19]. Considering the advantages of the application of erbium laser i.e. elimination of smear layer and creation of tooth surface irregularities, it appears that erbium laser may be able to decrease marginal microleakage.

Baghalian et al. showed that Er:YAG laser-prepared teeth restored with composite and Clearfil SE Bond, a one-step self-etch adhesive agent, demonstrated a better marginal seal at the occlusal and gingival margins compared with bur-prepared cavities [23]. Xie et al. reported that when all-in-one self-etch system was used after irradiation of Er:YAG laser, composite resin restorations showed less marginal microleakage [24].

Some studies have shown increased marginal microleakage of composite restorations after cavity preparation with Er:YAG laser [20,25]. Laser parameters can significantly affect the results and influence the marginal microleakage of composite restorations. Roebuck et al. reported that erbium laser with 240 mJ pulse energy; 5 Hz pulse repetition rate and 250 µs pulse duration decreased the microleakage at the enamel margins of restorations; whereas, in other pulse energies, no difference in microleakage was noted at the enamel or dentin margins compared to the control group [26]. Marotti et al. used Er, Cr, YSGG laser with different parameters for preparation and treatment of class V cavities and showed that only surface preparation with 5 W laser and pretreatment with 1 W laser decreased microleakage [9].

The current study revealed that long-term storage of specimens in water (six months) had no significant effect on microleakage at the dentin margins of the control, laser and CHX groups or the enamel margins of the control and CHX groups ($P>0.05$). In the current study, Bond Force one-step self-etch bonding agent was used. This bonding agent has a pH of 2.3 and is considered a mild self-etch (MSE) bonding agent; it contains acidic phosphate monomers [27,28]. In MSE bonding agents, dentin surface demineralization is not completely performed; thus, some of the hydroxyapatite crystals exposed to acidic monomers remain around the dentin collagen. These acidic monomers in self-etching adhesives are capable of forming ionic chemical bonds between the phosphate groups or the carboxylates with the calcium ions present in hydroxyapatite of the tooth structure and form a durable bond [29-31]. This mechanism can enhance the durability of bond to dentin over time and decrease microleakage. Inoue et al. stated that some active acidic monomers in self-etch adhesives not only form a strong chemical bond to hydroxyapatite, but also their bond to calcium is very resistant to hydrolysis during long-term thermo-cycling [32]. The ability of these acidic monomers to form an ionic bond to calcium is important; however, after formation, the bond must remain stable in an aqueous environment.

Moreover, the microleakage path enables the passage of fluids and their penetration into adhesive resin or the hybrid layer. This can result in extrusion of water-soluble uncured monomers and low molecular weight oligomers and lead to resin-dentin bond failure [30]. Nonetheless, a study reported that some parts of the etched dentin in the hybrid layer, where resin had no penetrated into, remineralized after six months [33]. Some others showed that remineralization occurs via the deposition of calcium phosphate crystals in adhesive porosities and the hybrid layer in partially demineralized dentin [30]. This enhances the durability of bond in long-term (one year) [30].

Further studies are required to optimize the concentration of CHX and find the most suitable laser parameters for dentin and enamel surface treatment prior to the use of one-step adhesive systems.

CONCLUSION

It was concluded from the results of the present study that, surface treatments with Er:YAG laser and 2% CHX prior to the application of Bond Force as a one-step self-etch adhesive agent had no effect on the marginal microleakage of class V composite restorations immediately or after 6 months of water storage. Water storage caused only an increase in enamel marginal leakage of the samples treated with Er:YAG laser.

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CONFLICT OF INTEREST

Authors declare there is no conflict.

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