Effect of Chlorhexidine and Ethanol on Microleakage of Composite Resin Restoration to Dentine

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Objective: *To evaluate the effect of chlorhexidine and ethanol on microleakage of composite resin restoration to dentine.*

Methods: *Class II cavities with dentinal margin were prepared on 96 premolar teeth. All specimens were acid-etched, rinsed and dried. Then the samples were randomly divided into four groups according to pre-treatment of the dentine: no treatment (control group); treatment with 100% ethanol for 60 s (group 2); treatment with 2% chlorhexidine for 60 s (group 3); 100% ethanol for 60 s and then 2% chlorhexidine for 60 s (group 4). After dentine treatment, each group was bonded and restored with a universal micro hybrid composite resin, according to the manufacturers' recommendation. Microleakage was evaluated by dye extraction method in two subgroups, immediately (24 h) and after 6 months in storage. Scan electronic microscope analyses for two samples of each group were also conducted. Data were analysed by two-way analysis of variance and Tukey test.*

Results: *The lowest and the highest amount of microleakage were observed in the ethanol group and in the control group, respectively. There were significant differences in microleakage among the groups ($P = 0.003$) and between measurement times ($P = 0.001$). For each storage time, the control group showed significant differences from the other groups and there were no differences between the other groups.*

Conclusion: *Ethanol-wet bonding and chlorhexidine application may have potential benefits in lowering the occurrence of microleakage in the long term.*

Key words: *chlorhexidine, ethanol, microleakage, surface treatment*

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Aesthetic demands for invisible restorations and awareness of the environmental implications of mercury in dental amalgam are driving the need for alternative tooth-coloured restorative dental materials over amalgam direct restorations. As with other materials, resin-based composite has its own limitations, the most significant problem being its durability following interfacial leakage. The risk of developing new decay around the margin of resin-based composite restorations is 3.5 times that of amalgam[1].

It was proposed that the reduction in bond strength is a consequence of hydrolysis of the resin polymer matrix and degradation of demineralised collagen[2]. To prevent this enzymatic and hydrolytic degradation, fully infiltrated demineralised dentine with resin is preferred[3]. However, using the conventional water wet-bonding technique cannot fulfil this objective.

Recently, ethanol wet bonding was introduced as a new bonding technique to promote resin infiltration of etch-and-rinse adhesives by replacing water in water-saturated demineralised dentine with ethanol and forming ethanol-saturated dentine[4]. Based on Hoy's

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solubility (δh) theory, ethanol-saturated dentine provides a higher degree of miscibility with hydrophobic and hydrophilic resins compared with water-saturated dentine. By replacing water with ethanol, ethanol-wet bonding reduces the hydrophilicity of demineralized dentine, thereby allowing hydrophobic resin to penetrate relatively hydrophobic ethanol-saturated dentine, and create a more integrated hydrophobic hybrid layer and, eventually, a more reliable bond[5]. Huang et al showed that dentine conditioning with ethanol increased the micro-tensile bond strength of resin to dentine, but had different effects on nano-leakage[6].

Meanwhile, chlorhexidine (CHX) plays a major role in preserving collagen integrity in the hybrid layer by its own matrix metalloproteinases (MMPs) inhibitor activity. It does this through two mechanisms: 1) interaction of CHX with the sulfhydryl group on domain and/or cysteine located at the active site of MMPs that may distort MMPs' molecules and prevent them from binding to substrates[7,8]; 2) competing for the binding of calcium and zinc ions to MMPs because of their chelating action. Without these ions, MMPs lose their catalytic activities[9]. Furthermore, CHX can create a long-term hybrid layer and bond consistency by avoiding the effect of the MMP enzyme in the degradation of collagen fibrils, whose resin has penetrated incompletely around them[10,11]. Stanislawczuk et al showed in an *in vivo* study that using CHX on acid-etched-dentine for 60 s could increase the durability of composite bond to tooth[12].

According to specific features of CHX and ethanol, the target of this study was to evaluate the effect of 2% CHX and ethanol on microleakage of resin composite restoration. Our hypothesis was that different methods of dentine conditioning would have no influence on immediate or delayed microleakage.

Materials and methods

In total, 96 intact human single-rooted maxillary premolars extracted for orthodontic reasons, with no visible decay or cracks, were collected after the donors' informed consent was obtained under a protocol approved by the Ethics Committee for Human Studies, Mashhad University of Medical Science, registry no. 920061. The teeth were cleaned and stored in a 0.1% thymol solution at 4°C for no longer than 1 month.

A box-only cavity was prepared on one of the proximal surfaces of all teeth. The cavities had 3 mm buccolingual width and 1.5 mm axial depth. The gingival margin was located 0.5 mm below the cemento-enamel junction. All preparations were accomplished with diamond fissure burs using a high-speed handpiece and cooled by air water spray. New burs were employed after every five preparations. The preparations were made as uniform as possible by a single operator.

The prepared teeth were randomly divided into four groups of 24 teeth per group, and restored as follows:

Group 1: Water wet-bonding technique (control)

The cavity preparations were etched with 35% phosphoric acid (Ultra-Etch 35%, Ultradent Products, South Jordan, UT, USA), applied to the enamel margins for 15 s and followed by the etching of dentinal walls for an additional 15 s before rinsing with water for 30 s. Excess water was removed from the dentine surface with absorbent paper. After placement of a Tofflemire stainless steel matrix (Teledyne-Water Pik, Fort Collins, CO, USA), two coats of Single Bond adhesive system (3M ESPE, St Paul, MN, USA) were applied to the cavity walls and light cured using a curing light (Blue phase C8, Ivoclar Vivadent, Schaan Liechtenstein) at a irradiance of 800 mW/cm^2 for 20 s. A universal micro hybrid composite resin (Filtek Z250, 3M ESPE) was incrementally inserted while each increment was approximately 2 mm in thickness and was light cured for 40 s. Finally, the restorations were polished with sandpaper discs (Soflex, 3M ESPE).

Group 2: Ethanol wet bonding technique

The process was the same as group 1, except that following acid etching, the cavity walls were saturated with 100% ethanol for 60 s and then dried with cotton balls. This step was followed by Single Bond application, as for the first group.

Group 3: Surface conditioning with CHX

This followed the same process as group 1, except that following acid etching, an aqueous solution of 2% CHX (Concepsis, Ultradent Products) was applied for 60 s, followed by air drying. This step was followed by Single Bond application, as for the first group.

Group 4: Surface conditioning with ethanol wet-bonding technique and CHX

The process was the same as for group 1, except that following acid etching, the cavity walls were saturated with 100% ethanol for 60 s and then dried with cotton balls. After that, aqueous solution of 2% CHX (Concepsis, Ultradent Products) was applied for 60 s. This step

was followed by Single Bond application, as for the first group.

In each group the specimen were divided in to two subgroups. Half of the specimen in each group were stored in an incubator at 37°C and 100% humidity for 24 h for immediate assessment of the microleakage, while the rest were stored in an incubator at 37°C and 100% humidity for 6 months to evaluate the delayed microleakage.

Dye extraction test:

After the storage period, dye extraction technique was used to evaluate the amount of microleakage. According to a study by Youngson[13], 10 specimens from each group were used for microleakage assessment and the other two samples used for scanning electron microscopic (SEM) evaluation.

The specimens were prepared by applying two coats of nail varnish on the teeth within 1 mm of the gingival margins. They were then immersed in 2% methylene blue for 48 h and rinsed under tap water for 30 min. The nail varnish was removed with sandpaper discs. The root of each tooth was sectioned just below the dye penetration region and then the remained crown was stored in a vial containing 1,000 µl of concentrated (65 wt%) nitric acid for 3 days. Vials were then centrifuged (Versatile SIGMA 2-16P centrifuge, Montreal Biotech, Montreal, Quebec, CA) at 14,000 rpm for 5 min and 100 µl of supernatant from each sample was transferred to an automatic micro-plate spectrophotometer (CECIL Instruments, Cambridge, UK). The samples were read at 550 nm using concentrated nitric acid as the blank[14].

The results of the spectrophotometer indicate the light absorption of the methylene blue in the resin-dentine interface, which is actually showing the microleakage of the restoration.

Scan electronic microscope (SEM) evaluation:

Teeth were sectioned mesiodistally through the centre of the restoration to achieve a sample thickness of 2 mm. The sections were polished by using 400, 600, 800, 1,000, 1,200 grit silicon carbide abrasive paper, respectively, and then demineralised in hydrochloric acid solution (6 mol/L) for 10 s and deproteinated in 1% sodium hypochlorite solution for 10 min. Next the specimens were ultrasonicated and immersed in 30, 50, 70, 80, 85, and 95% ethanol, and three times in 100% ethanol for 10 min respectively, in order to desiccate them. Finally they were sputter-coated with gold and examined in a SEM (LEO 1450, Zeiss, Oberkochen, Germany), using an accelerating voltage of 30.0 kV. Under SEM, samples were photographed using 2,500× magnification (Figs 1 to 4) and gaps were measured using image analyser software. At two points of minimum and maximum gap formation the analyser reported the size of the gap.

Statistical analysis

Statistical analysis for microleakage was performed using two-way analysis of variance (ANOVA) by SPSS version 14.0 (SPSS, Chicago, IL, USA). The Tukey test was carried out for pair-wise comparison between the means when the ANOVA was found to be significant. The significance level was set at $\alpha = 0.05$.

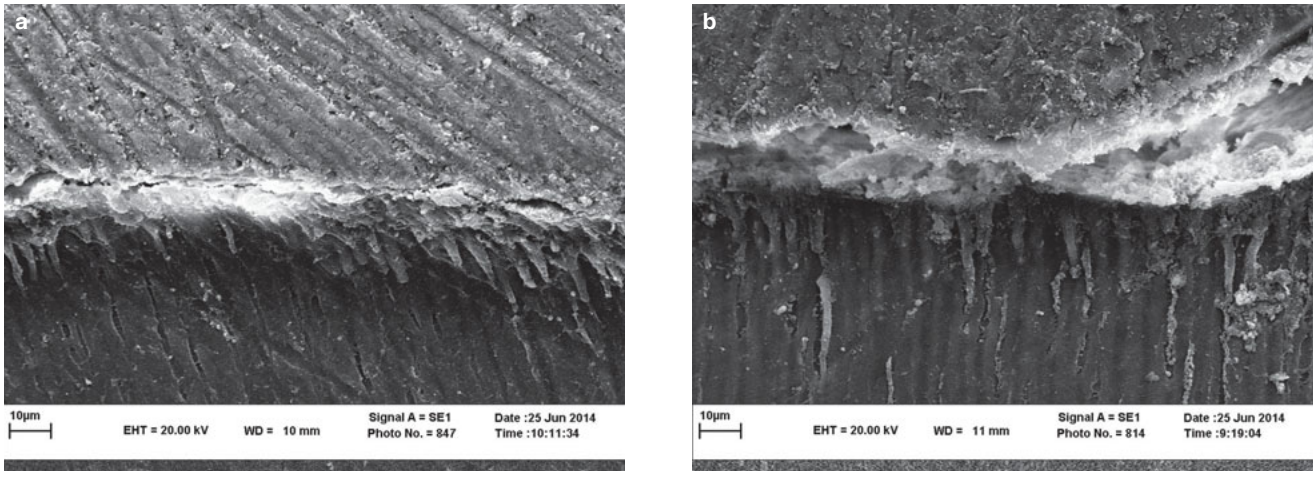

Fig 1 a) SEM evaluation of the control group after 1 day storage; and b) after 6 months' storage.

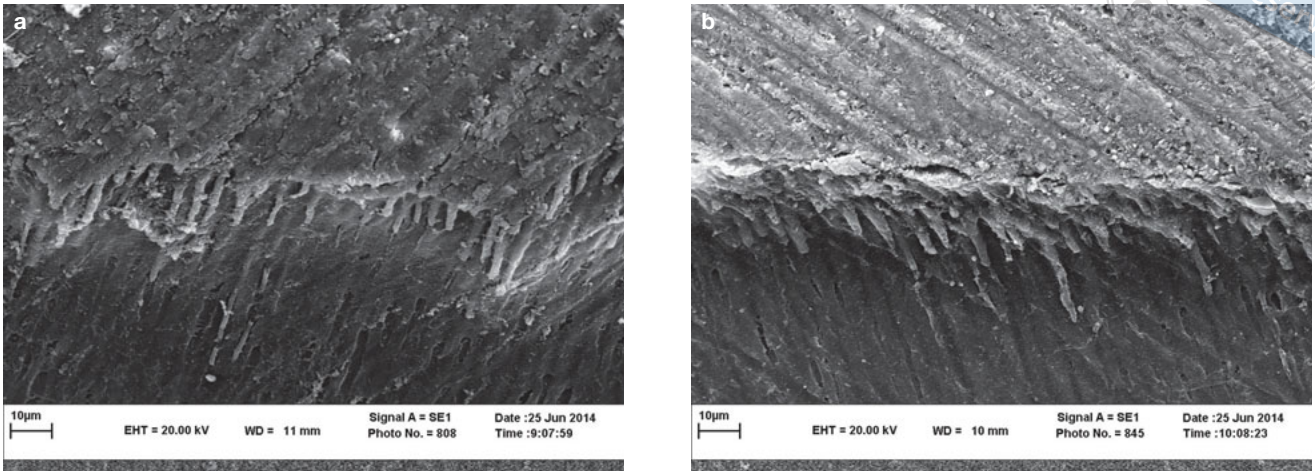

Fig 2 a) SEM evaluation of the ethanol group after 1 day storage; and b) after 6 months' storage.

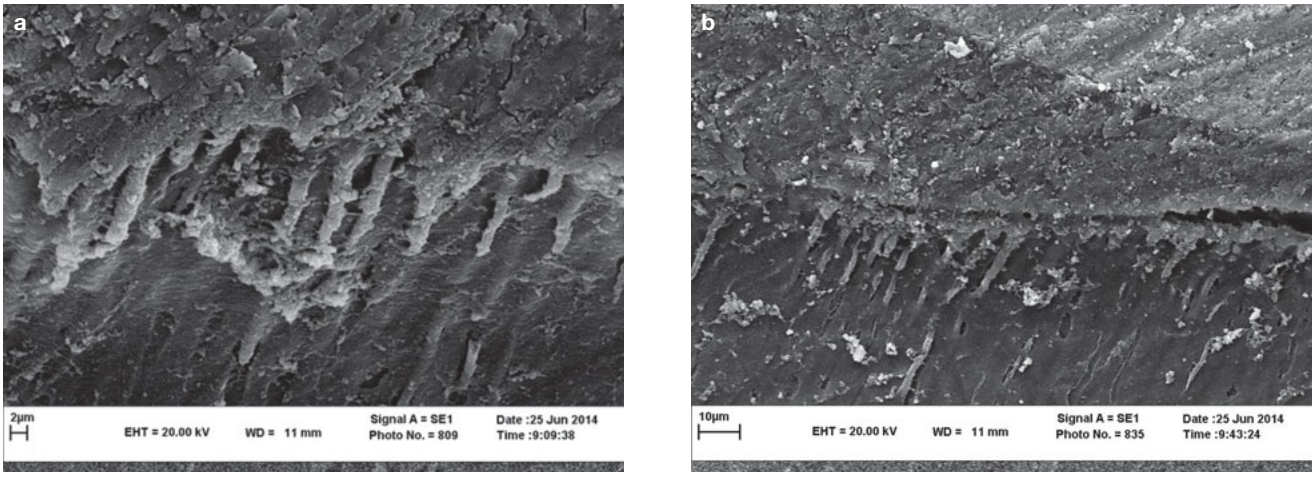

Fig 3 a) SEM evaluation of the CHX+ethanol group after 1 day storage; and b) after 6 months' storage.

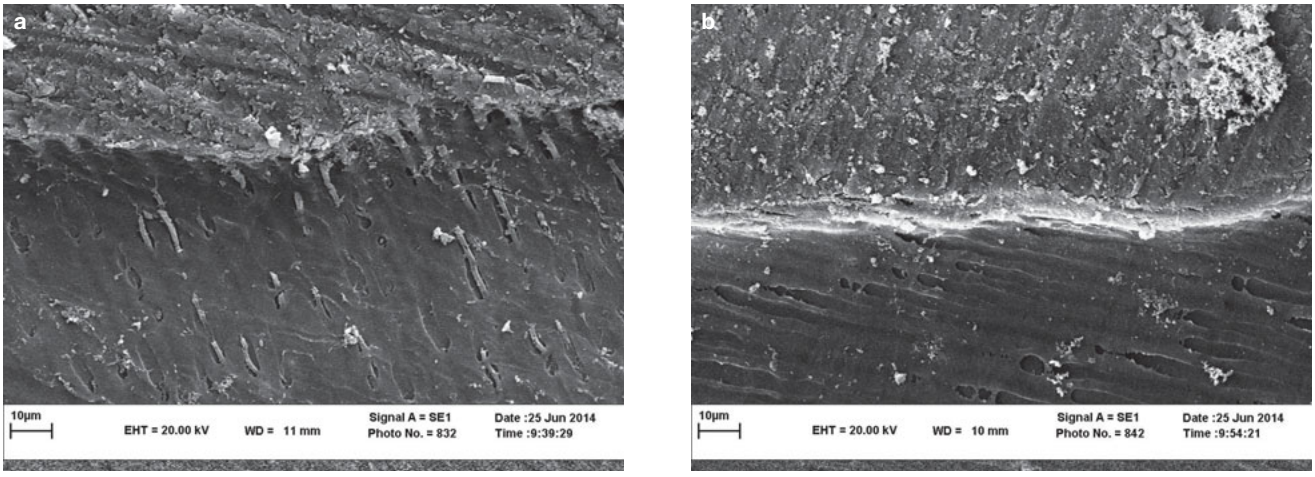

Fig 4 a) SEM evaluation of the CHX group after 1 day storage; and b) after 6 months' storage.

Results

All restoration techniques showed some degree of microleakage. The mean microleakage of all groups is presented in Table 1. The highest amount of immediate and delayed microleakage related to the control group and the least amount to group 2 (ethanol wet-bonding technique). Statistical analysis showed a significant difference between group 1 and groups 2, 3 and 4 ($P = 0.001$), and there were no significant differences between the other groups in immediate or delayed microleakage. Within groups, statistical analysis showed a significant difference between delayed and immediate microleakage in all groups in such a way that after six months, the leakage rate increased significantly compared with the first day ($P = 0.003$).

Interfacial gaps between dentine and the adhesive layer were measured. The mean interfacial gap values for group 1 were the highest, whereas group 2 showed the least value among the tested groups. The mean interfacial gaps values are presented in Table 2.

Discussion

The findings of the present study showed a significant difference between the micro-leakage of group 1, and groups 2, 3, and 4. In addition, the micro-leakages after a 6-month storage interval in all groups were more than the immediate micro-leakage.

Different leakage-testing methods have been used to assess the ability of materials to seal the margin of restorations, including fluid filtration, dye penetration, dye extraction, and bacterial leakage models. Campes and Pashley showed that the dye extraction method yielded the same results as fluid filtration[15]. The dye extraction technique is a quantitative measuring tool and it shows the three-dimensional characteristic of micro-leakage[16]. This study showed that regardless of time, using CHX, ethanol, or CHX after ethanol, significantly decreases the leakage rate in comparison with the control group. However, the ethanol group had a better result.

Different studies showed that using CHX for 1 min on an etched dentine surface, after removing acid etching in the total-etch dentine bonding system, is an effective technique for increasing the durability of the dentine-resin bonding interface. CHX is soluble in water. It dissolves in physiological pH, hence, it is absorbed by the smear layer and makes the dentine surface resistant to acid. When strong acids are used in dentine, a demineralised dentine zone is created within the bonding structure by the incomplete diffusion of monomers into the collagen network. The lack of full penetration has a negative influence on collagen[17]. In addition, MMP enzymes that originate from the dentine are activated after acid etching. De Munck et al showed that CHX is effective on the durability of tensile bond strength only in total-etch adhesives[9]. Brackett et al used the Single-bond adhesive to gain the maximum desired result of CHX and showed the compatibility of CHX with the Single bond[18].

A stable hybrid layer after washing acid-etched dentine with CHX is due to the ability of CHX to crosslink the collagen of the dentinal matrix. Regardless of the volume and the time of CHX application, a significant amount of it remains in the dentinal matrix[19]. Some researchers who previously investigated the effect of CHX on the bond strength of dental adhesive systems on dentine have reported that the CHX pre-treatment

Table 1 The mean micro-leakage of different test groups.

Group/time	24 h	6 months
Control (group 1)	0.63 ± 0.13^{a}	0.78 ± 0.13^{c}
Ethanol (group 2)	0.44 ± 0.14^{b}	0.53 ± 0.1^{d}
Chlorhexidine (group 3)	0.53 ± 0.15^{b}	0.6 ± 0.15^{d}
Ethanol + Chlorhexidine (group 4)	0.46 ± 0.15^{b}	0.55 ± 0.2^{d}

Different superscript letters (a, b, c or d) indicate statistical difference between the test groups ($P < 0.05$).

Table 2 The mean interfacial gaps of different test groups.

Group	24 h	6 months
Control (group1)	14-15 μm	15-30 μm
Ethanol (group 2)	0.82-2.9 μm	5.9-8.0 μm
Chlorhexidine (group 3)	2.5-1.5 μm	7.0-12.5 μm
Ethanol + Chlorhexidine (group 4)	2.0-3.5 μm	10-15 μm

did not affect the shear bond strength of dental adhesives[20,21]. Nevertheless, other studies have reported that CHX acting as the inhibitor of MMPs decelerates the rate of resin-dentine bond degradation[22,23]. In a previous study conducted by Campos et al, the preservative effect of CHX on the bond strength of etch-and-rinse adhesives was reported during a 6-month aging period[24]. In another study, extensive degradation was observed in all of the teeth in the control group after 12 months, while no degradation was observed in the CHX group[25]. Generally, different studies show that a decrease in bond consistency in the control group occurs in between 1 and 5 months after restoration, while in the CHX group this degradation occurs 10 to 12 months after restoration[26,27]. In our study, the CHX group showed a lower increase in the leakage rate after 6 months compared with other groups. Hajizadeh et al showed that CHX could decrease tooth hypersensitivity after restoration[28]. Actually, tooth hypersensitivity after restoration could be a clinical sign of leakage and bond degradation.

The ethanol wet-bonding technique can be used in two different ways. In the first, simpler way, pure ethanol is applied on the acid-etched dentine for 1 min. This technique is clinically practicable, but it is also technique-sensitive and removes all the remaining dentinal water at the same time. In the other method, ethanol of different purities is applied step-by-step on the acid-etched dentine. Therefore, the water in the collagen matrix is removed gradually. This is a time-consuming technique and is not preferred clinically[29]. We use the first technique in this study, so that in the case of positive results, it would be a practical option for use in the clinic. Upon application of ethanol on water-saturated dentine, 18% shrinkage of the collagen matrix occurred. Ethanol-saturated dentine allowed a small degree of new inter-peptide H-bonding formation through the exchange of higher δh water with lower δh ethanol. These new inter-peptide H-bonds might be responsible for shrinkage and an increase in the stiffness of the collagen matrix. The stiffness of ethanol-saturated dentine might prevent collagen collapse during a subsequent adhesive application[30]. Moreover, ethanol with high water vapour may eliminate most of the water within the hybrid layer. The limited amount of water left in the collagen matrix may reduce resin phase separation, thus resulting in more homogeneous polymerization of the resin matrix; hence, a durable bond is speculated[31]. In this study, the ethanol group showed a lower increase in the leakage rate after 6 months compared with the control group.

Leakage tests are used to evaluate the marginal seal and the quality of the hybrid layer by assessing subsurface adaptation through evaluating microleakage at the bonding interface[18]. There was a general trend toward higher bond strength causing less leakage[17]. Shin et al[32] and Huang et al[6] showed that ethanol wet-bonding increases resin-dentine bond durability in a short period. Li et al[33] and Talungchit et al[34] showed that μ tensile bond strength of the ethanol pretreatment group is higher than other groups immediately and after storage. This confirmed the results of present studies indirectly. Besides, Pei et al[35] showed that surface preparation with ethanol before Scotchbond adhesive had no effect on micro-tensile bond strength. Of course, in the mentioned study, the technique of ethanol usage was different from that used in the present study.

In group 4, where both CHX and ethanol were applied, better results were expected because of the positive effects of both substances. But the results were almost the same with the application of CHX or ethanol alone (groups 2 and 3), and the application of CHX and ethanol together had no superiority. Ekambaram et al[36] have concluded that adding 2% chlorhexidine to ethanol-wet bonding did not further improve the bonding of a fibre post to intraradicular dentine, compared with ethanol-wet bonding alone after 12 months of ageing.

In addition, SEM evaluation and measuring the size of interfacial gaps between dentine and the bonding layer confirmed the results of the dye extraction method. The size of the gap increased after 6 months and it was the least in the ethanol group. Different studies showed that the size of interfacial gaps between dentine and the bonding layer could be the size of a single hair, about 150 μl for composite restorations. Consequently, from a clinical point of view, all the groups showed a gap within the acceptable range in 6 months.

Conclusion

This study shows that conditioning the dentine surface after acid etching with ethanol or CHX, individually or with each other, can be effective in decreasing the leakage, especially over time. As the use of CHX and ethanol together needs too much time, it is recommended that at least one of these materials be used during composite resin restorations.

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Conflicts of interest

The authors reported no conflicts of interest relating to this study.

Author contribution

Dr Iman Ramezanian Nik collected, analysed and interpreted data, and final approved the manuscript; Dr Ehsan Baradaran Naseri designed the study and finally approved the manuscript; Dr Sara Majidinia designed the study and prepared the manuscript; Dr Sara Ramezanian Nik collected, analysed and interpreted data; Dr Mohammad Jafari Giv drafted the manuscript.

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