Original Article

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Effect of ethanol wet bonding technique on bonding performance of dual-cured adhesive to root canal dentin

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Objectives: To evaluate the push-out bond strength and interfacial nanoleakage of root canal dentin regions with various ethanol-wet bonding techniques using dual-cured etch-and-rinse adhesive and core build up resin composite in 24 hour storage and 10,000 cycles of thermocycling

Materials and Methods: Roots of forty-eight extracted human premolars were prepared to 15 mm root length. Post space were prepared using FRC Postec® Plus reamer size 1 to a depth of 10 mm. All root canals were etched by 37% phosphoric acid and then divided to 4 groups (N = 12 per group) according to ethanol-wet bonding applications: Control [C], 70% ethanol [E70], 100% ethanol[E100] and Stepwise application [S]. The ethanol solution was achieved into root canal with 1 ml of each solution for 60 s. For the stepwise ethanol application, 50%, 70%, 80%, 95% and 100% in 3 times were performed for 30 s in each application. The treated root canal dentin surfaces were applied with ExciTE® DSC and Multicore® flow. Then, light curing for 40 s was performed. After water storage at 37ºC for 24 hours, the half of specimens in each group (N=6) were thermocycled for 10,000 cycles. The bonded roots were horizontally sections into 1- mm thick slab. In each group, 3 slabs from coronal part and 3 slabs from middle part were selected to evaluate the nanoleakage. Then, the 15 slabs were subjected to push-out bond strength testing. The failure mode was observed under SEM. The push-out bond strength values were statistically analyzed by ANOVA and Tukey's test. The failure pattern was statistically analyzed by Pearson Chi-Square test (α=0.05). Descriptive analysis was used to interpret the nanoleakage evaluation.

Results: The push-out bond strength of E100 group and S group were significantly higher than these of C group and E70 group for all storage times and regions. At 24 hours storage time, the push-out bond strength of E100 group and S group at middle region of post space was significantly higher than that at coronal region. The mixed failure was more prominent in E100 group and S group, while C group and E70 group with both storage times were mostly the adhesive failure. There was no silver nitrate deposition along the hybrid layer in S group at 24 hours and after thermocycling group.

Conclusions: The stepwise application of ethanol-wet bonding with dual-cured etch-and-rinse adhesive improved the bonding and bond durability of resin core material to root canal dentin.

Keywords: bonding interface, dual-cured adhesive, ethanol, ethanol-wet bonding, push-out bond strength, nanoleakage

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Introduction

Recently, minimally invasive restorations of endodontically treated teeth have been feasible by the use of dentin bonding systems and composite resin. Fiber post or fiber-reinforced composite post has become commonly to be used for restoration of endodontically treated tooth with minimal coronal tooth loss [1]. Fiber posts are luted into root canal using various resin cements or resin composite and dental adhesives.

For restoration of endodontically treated tooth, the failure of bonding in intraradicular dentin is mainly from debonding at the dentin resin composite interface [2], as a result of resin dentin bond degradation [3]. Moreover, the attenuated light penetration into deeper part of post space or root canal have been reported to reduce bonding efficacy [4, 5]. Therefore, dual-cured adhesive systems, a rapid light polymerization in the areas where curing light penetrates effectively and a slower chemical polymerization in the areas where the light is not effective; have been introduced to solve the attenuated light problem in root canal [6].

On the other hand, removal of water remnant in deeper part of root canal dentin is also difficult [6]. Therefore, the ethanol-wet bonding technique is the choice for water removal in deep part of root canal dentin. This technique improves longevity of resin-dentin bonds by preventing water sorption from hydrophilic adhesives [7-9]. Ethanol has been introduced to replace water and supported the demineralized dentin collagen fibrils [10]. Recently, the ethanol-wet bonding had also been used in root canal dentin [11]. However, there is no any consensus about the ethanol concentration for this technique. And also, there was no study about the ethanol application in one concentration to root canal dentin for dentin dehydration before applying 2-step etch-and-rinse adhesive. Therefore, this study aimed to evaluate the push-out bond strength and interfacial nanoleakage in various techniques of ethanol-wet bonding application to intraradicular dentin when dual-cured etch-and-rinse adhesive with dual-cured core build up resin composite were performed after 24 hours and 10,000 cycle of thermocycling. It may provide the proper protocol of ethanol-wet bonding in clinical situation.

Materials and methods

Preparation of test specimens

Forty-eight human single-rooted, single canal premolars were used in study. The teeth were stored in a 0.1% thymol solution. The teeth were decoronated at the cemento-enamel junction using slow speed diamond disc under water cooling. The 15 mm root length was obtained. The post space were prepared by using a size 1 of FRC Postec® Plus reamer (ø 0.7 mm, Ivoclar Vivadent, Schaan, Leichtenstein) with a low speed hand piece under copious water irrigation to a depth of 10 mm. All root canal dentin were etched with 37% phosphoric acid for 15 seconds, and then rinsed off with water for 20 seconds and dried with absorbent paper point to keep the visibly moist surfaces. The prepared roots were randomly divided into 4 groups (12 roots in each group), the control group (Group 1), the specimens were left moist with distilled water and without ethanol treatment. In the experimental groups, the specimens were treated as follow, Group 2 with 70% ethanol for 60 seconds, Group 3 with 100% ethanol for 60 seconds and Group 4 with stepwise ethanol application protocol. After applying the phosphoric acid into post space, the ethanol-wet bonding substrate was achieved by occupying the post space with 1 ml of 70% and 100% ethanol solution for 60 seconds. For the stepwise ethanol application, serial ethanol solutions (50%, 70%, 80%, 95%) were used in 1ml for 30 seconds in each fourth solutions and 1 ml of 100% ethanol was then applied for 30 seconds with 3 times. After that, all root canal dentins in post space were dried by using absorbent paper points (Dentsply DeTrey, Konstanz, Germany).

For bonding procedure, the dual-cured adhesive (ExciTE® F DSC, Ivoclar Vivadent, Schaan, Leichtenstein) was applied with light scrubbing motion at least for 10 seconds. A gentle air blowing from triple syringe for 10 seconds with 1 cm distance from the post orifice was applied to disperse the adhesive to be a thin layer. Excess adhesive in post space was then removed by using absorbent paper point for 10 second. The solvent in adhesive was evaporated using gentle air blowing for 10 seconds [12]. A dual-cured core build up resin composite (Multicore® Flow) were filled into bonded post space using a centrix syringe (Centrix, CT, USA) and then light cured for 40 s with 1,000 mW/cm^2 of light intensity (Bluephase®,Ivoclar Vivadent, Schaan, Leichtenstein).

All bonded specimens were stored in distilled water at 37°C for 24 hours. Prior to push-out bond strength test, half of the specimens in each group (N=6) were thermocycled for 10,000 cycles in deionized water from 5 to 55°C (Thermocycler TC 400, King Mongkut's institute of Technology Ladkrabang, Thailand). The dwell time at each temperature was 15 s in each bath and the transfer time between the water baths is 10 seconds.

Push-out bond strength test

The forty-eight bonded roots (6 root canal dentins in each group) were horizontally sectioned, which were perpendicular to the long axis of root, into 1-mm thick slab under water cooling (Isomet™, Buehler Ltd., Lake Bluff, IL, United States). Three slabs from the coronal part of root canal dentin (n=18) and three slabs from the middle part of root canal dentin (n=18) were obtained. The void or gap in resin core material was observed under light microscope at 10x. If the void or gap was detected, that specimen was excluded. The middle slab of coronal and middle part of root canal dentin regions were selected for nanoleakge evaluation (n=3). Then, the remained specimens (n=15) were subjected to push-out bond strength testing. The push-out load was applied using cylindrical plungers attached to a universal testing machine (EZ test, Shimadzu, Japan). The apical side of slab was positioned facing the punch tip. The loading force was applied in an apical-coronal direction with crosshead speed of 0.5 mm/min until failure. Push-out bond strength were converted into magapascals (MPa) by dividing the load at failure in newtons (N) by the bonded surface area (S_L) in mm^2. Where, S_L were calculated at the lateral surface of a truncated cone using formula.

$$S_L = \pi(R+r)\,[(h^2 + (R-r)^2]^{0.5}$$

Where 'R' is the coronal core build up composite radius, 'r' is the apical core build up composite radius, and 'h' is the thickness of the slab. The diameters of the core build up composite and the thickness of the slab was measured using a digital caliper with 0.01 mm accuracy.

After push-out bond strength tested, specimens were cut in the middle half of slab to assess the failure mode using a SEM (JSM-6610LV, JEOL Ltd., Tokyo, Japan). The failure mode was classified as this following

(i) Adhesive failure between dentin and resin composite core build up (≥60%)

(ii) Cohesive failure within resin composite core build up or dentin (≥60%)

(iii) Mixed failure (adhesive and cohesive fractures occurred simultaneously; each pattern will be shown < 60%)

The fractured specimens were coated with gold and observed under scanning electron microscope at magnification of 60.

Nanoleakage evaluation

The six slabs in each group from the middle slab of coronal and middle part of root canal dentin regions in bonded roots for push-out bond strength test were used for interfacial nanoleakage analysis after 24 hours and 10,000 cycle of thermocycling. The slabs were cut to be half of slab and coated with two layers of fast-drying nail varnish, except the 1 mm window of the bonded interfaces on the inner surface of the specimen. After rehydration in distilled water for 10 min, the vanish-coated tooth slabs were immersed in 50 wt% ammonium silver nitrate ($AgNO_3$) solution (pH 9.5) in the dark for 24 hours. The silver-impregnated slaps were rinsed with de-ionized water for 5 min, followed by placing of the slabs in a photo-developing solution under a fluorescent light for 8 hours to facilitate the reduction of silver nitrate ions into metallic silver grains [13] and then fixed for 2 minutes. After that, the specimens were embedded in epoxy resin in PVC ring and the embedded sections were polished with SiC paper and then fine diamond paste (6μm, 3μm, 1μm and 0.25μm). The specimens were cleaned ultrasonically and air dried. All specimens were dehydrated in a desiccator for 24 hours. The dried specimens were mounted on aluminium stubs and sputter-coated with gold. Examination is then carried out with the scanning electron microscope in backscatter mode at the constant magnification of 1,000 and 2,000.

Statistical analysis

The push-out bond strength data were analyzed using a statistical package (PASW statistic 18, SPSS Inc., Chicago LA). Three-way ANOVA was used to examine the effect of ethanol-wet bonding techniques, root canal dentin regions and storage times. Tukey HSD's multiple comparison test was used to determine the statistical differences among the mean push-out bond strength in each group at the level of significance as $\alpha < 0.05$.

A failure mode was statistically analyzed by nonparametric Pearson chi-square test.

Descriptive analysis was used to interpret the SEM images of the nanoleakage evaluation.

Results

Evaluation of push-out bond strength

Push-out bond strength to root canal dentin region

A significant effect of two independent factors; ethanol-wet bonding technique and root canal dentin regions were demonstrated with a significant of $p < 0.001$. The interaction among independent factors was not observed ($p < 0.05$).

The mean and standard deviation of push-out bond strength were demonstrated in Table 1 and 2. For 24 hours storage, the push-out bond strength of control group and ethanol 70% group (E70) were significantly lower than ethanol 100% group (E100) and stepwise technique group in all regions. In ethanol 100% group (E100) and stepwise technique group, the push-out bond strength at middle root canal dentin was significantly higher than that in coronal root canal dentin. After 10,000 cycles of thermocycling, the push-out bond strength of all experimental groups was similar to the result from 24 hours storage in all regions. At the middle part of root canal dentin region, the push-out bond strength of stepwise group was significant higher than ethanol 100% group, ethanol 70% group and control group. Thus, the null hypothesis was partially rejected.

The upper-case letter indicates the middle part of root canal dentin and the lower-case letter indicates the coronal part of root canal dentin. Within the same column, different letters indicate significant difference in strategies ($p<0.05$), while the "*" symbol indicates significant difference in region within the same row, ($p<0.05$)

Table 1 Push-out bond strength (Mean, SD; MPa) to root canal dentin region with different strategies at 24 hour storage

	Strategy	Region					
		Coronal			Middle		
		Mean	SD	Sig	Mean	SD	Sig
24 h storage	Control	7.04	1.67	a	8.94	4.29	A
	E70	6.93	3.24	a	9.09	2.99	A
	E100	10.47	3.08	b	13.78	3.16	B*
	Stepwise	12.73	3.13	b	15.09	2.67	B*

Table 2 Push-out bond strength (Mean, SD; MPa) to root canal dentin region with different strategies after 10,000 cycles of thermocycling

	Strategy	Region					
		Coronal			Middle		
		Mean	SD	Sig	Mean	SD	Sig
10,000 cycles	Control	6.65	2.25	a	8.44	3.79	A
	E70	7.07	1.76	a	7.65	1.88	A
	E100	11.83	2.041	b	11.84	1.84	B
	Stepwise	12.23	3.44	b	14.14	3.78	C

Failure modes

Pearson Chi - Square test was performed to statistically analyze the percentage of each failure mode. It demonstrated significant difference in the failure mode in coronal part of stepwise group (p=0.002) and middle part of 70% ethanol group (p=0.001) in all storage times. The distribution of failure modes for all groups was shown in Table 3 and Figure 1. In 24 hours storage group, the most failure was the adhesive failure. However, in the stepwise group in both regions, the mixed failure was prominent. Obviously, when comparing to control group, the increase of mixed failure was shown in E70, E100 and stepwise group, respectively. In 10,000 cycles of thermocycling group, almost groups presented in adhesive failure, except stepwise group in both root canal dentin regions showed in mixed failure.

Evaluation of micromorphology of resin-tooth interfaces

The silver nitrate was used as a trace element to detect nanoleakage within the resin-dentin substrate interfaces under SEM. The representative SEM images of silver nitrate accumulation as nanoleakage at the resin-root canal dentin interfaces for the experimental conditions were demonstrated in Figure 2 - 9.

The deposition of silver nitrate could be observed at 24 hours and 10,000 cycles of thermocycling, except in stepwise group. The control group showed deposition of silver nitrate along the adhesive layer at 24 hours and 10,000 cycles of thermocycling. On the other hands, the 70% and 100% ethanol groups showed deposition of silver particles as nanoleakage within the interfaces along the bottom of hybrid layer. Interestingly, no silver depositions were found in the stepwise group at 24 hours storage and 10,000 cycles of thermocycling. (Figure 5 and 9)

Table 3 Failure mode distribution: comparison of failure mode distribution among different bonding strategies and root canal dentin regions. Adhesive failure (A) / Cohesive failure in resin composite core build up and dentin (C) / Mixed failure (M)

	Strategies	Failure Mode (Percentage)					
		Coronal (A/C/M)			Middle (A/C/M)		
		A	C	M	A	C	M
24 h storage	Control	93.33	0	6.67	93.33	0	6.67
	E70	80	0	20	53.33	0	46.67
	E100	46.67	20	33.33	26.67	20	53.33
	Stepwise	13.33	0	86.67	0	20	80
10,000 cycles	Control	100	0	0	100	0	0
	E70	100	0	0	100	0	0
	E100	60	20	20	80	0	20
	Stepwise	33.33	40	26.67	26.67	13.33	60

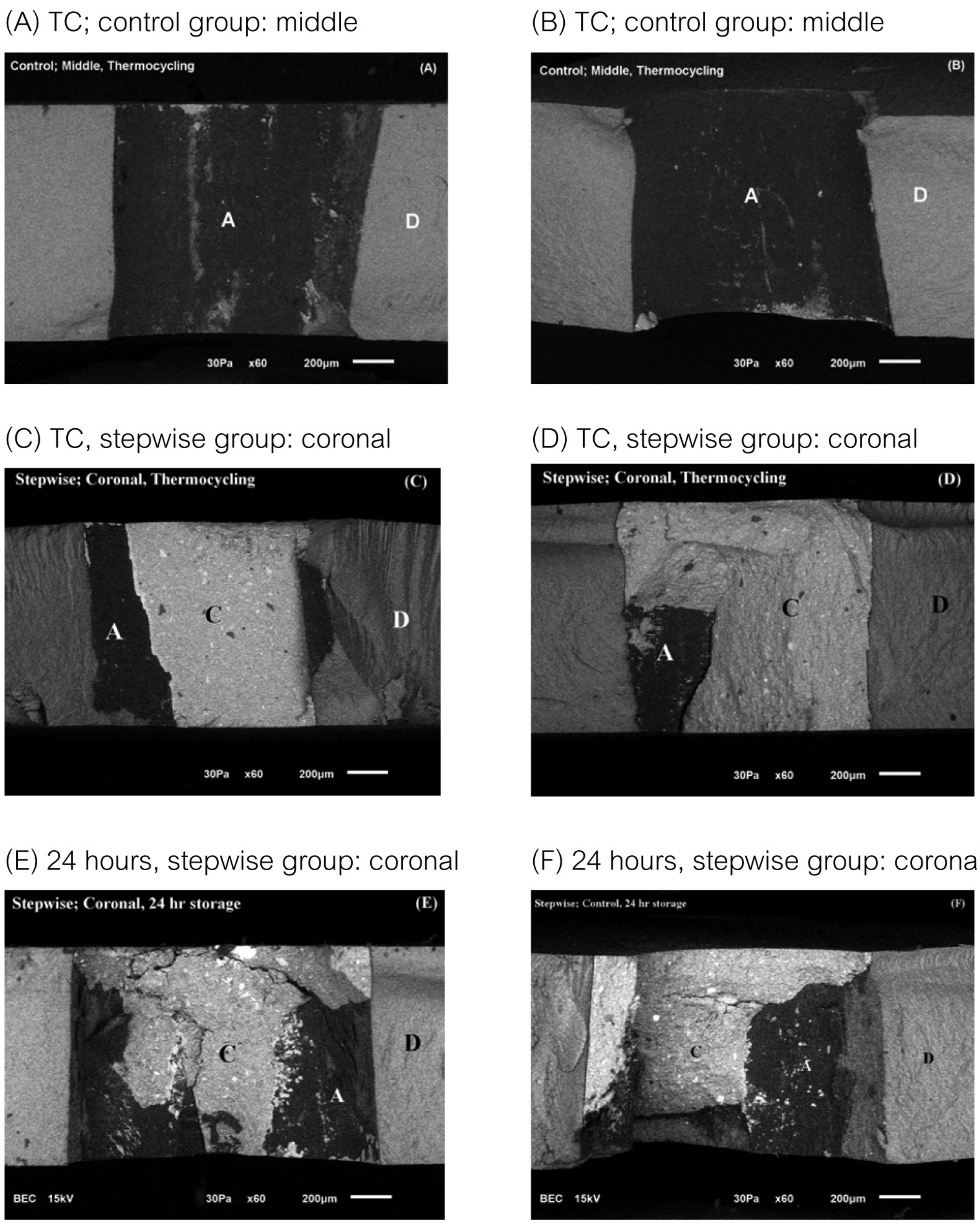
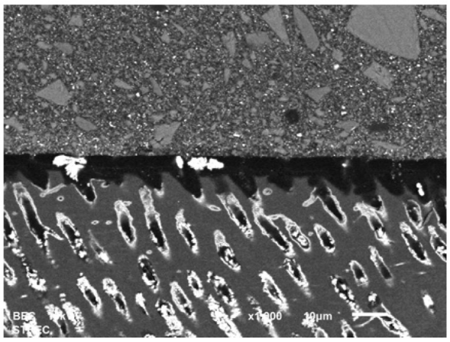
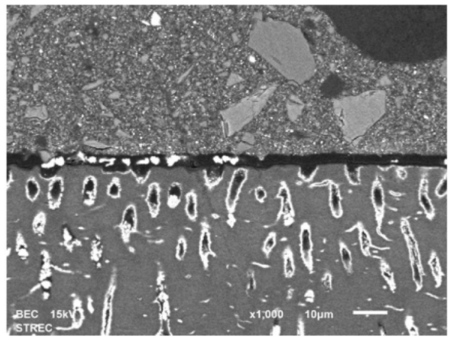


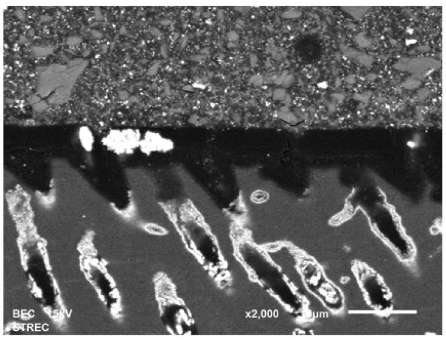
Figure 1 Representative SEM phtotographs of failure modes; (A, B) adhesive failure of thermocycling control group in middle part of root canal dentin region, (C, D) cohesive failure in thermocycling stepwise group in coronal part of root canal dentin region, (E, F) mixed failure in 24 hours storage stepwise group in coronal part of root canal dentin region



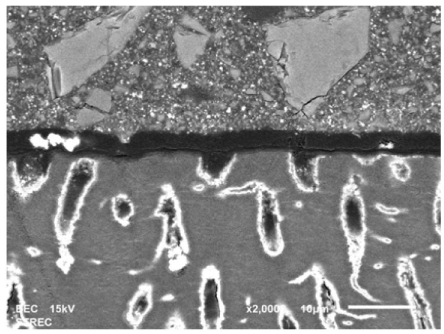
(a) Coronal (1000x), 24h storage



(b) Middle (1000x), 24h storage

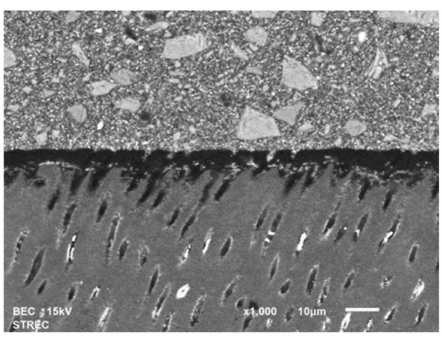


(c) Coronal (2000x), 24h storage

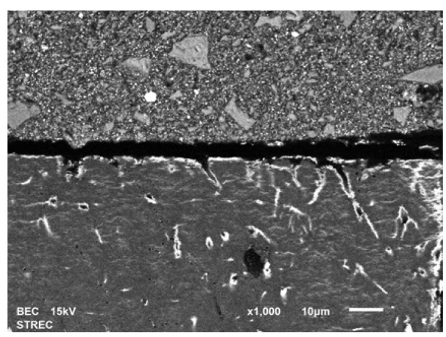


(d) Middle (2000x), 24h storage

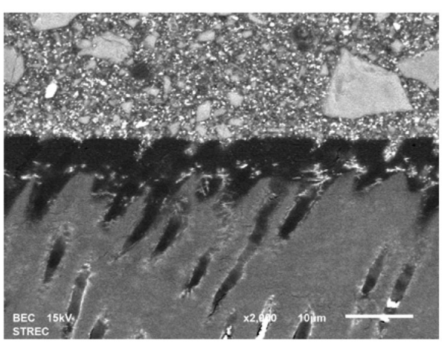
Figure 2 Representative SEM photographs of nanoleakage in control group (1000x, 2000x) with 24 hours storage at the coronal (a, c) and middle part (b, d).



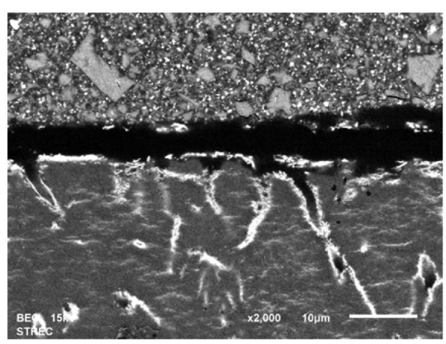
(a) Coronal (1000x), 24h storage



(b) Middle (1000x), 24h storage

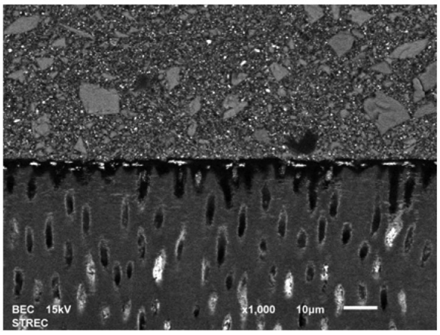


(c) Coronal (2000x), 24h storage

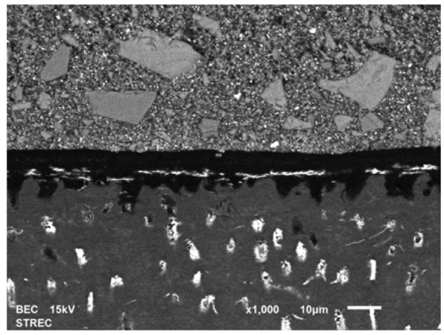


(d) Middle (2000x), 24h storage

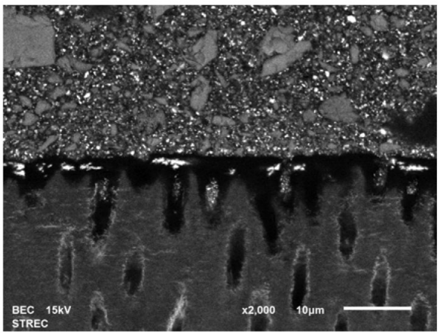
Figure 3 Representative SEM photographs of nanoleakage in ethanol 70% group (1000x, 2000x) with 24 hours storage at the coronal (a, c) and middle part (b, d).



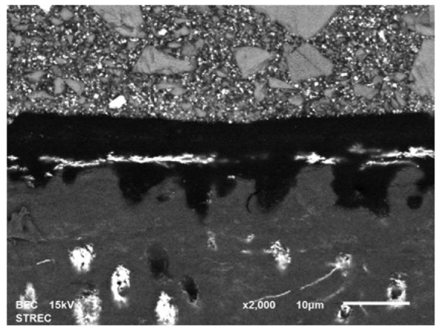
(a) Coronal (1000x), 24h storage



(b) Middle (1000x), 24h storage

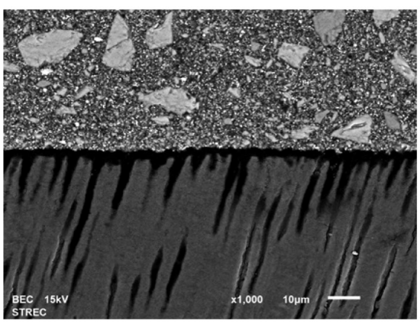


(c) Coronal (2000x), 24h storage

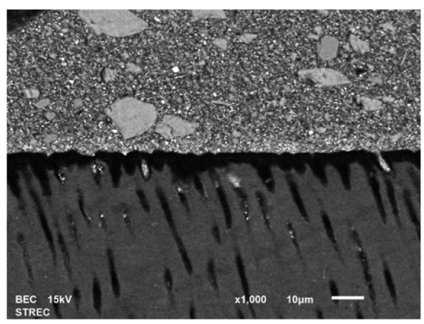


(d) Middle (2000x), 24h storage

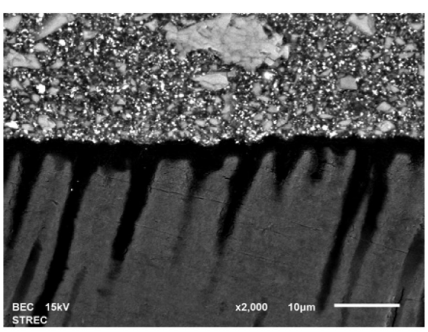
Figure 4 Representative SEM photographs of nanoleakage in ethanol 100% group (1000x, 2000x) with 24 hours storage at the coronal (a, c) and middle part (b, d).



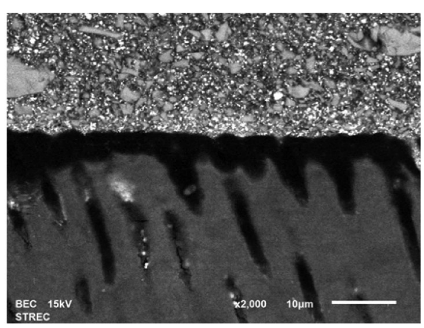
(a) Coronal (1000x), 24h storage



(b) Middle (1000x), 24h storage

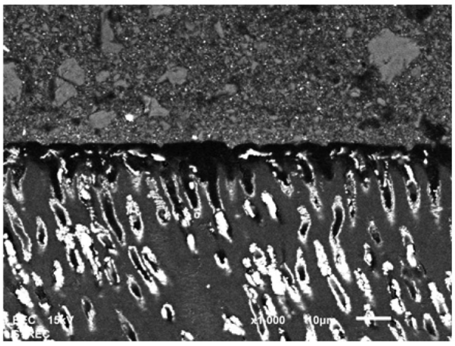


(c) Coronal (2000x), 24h storage

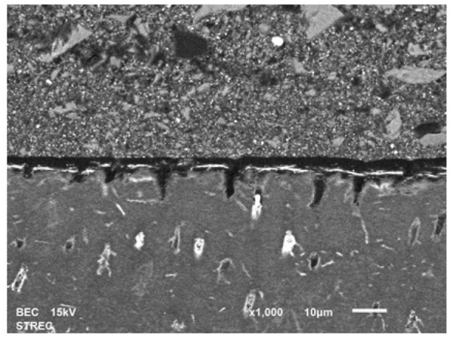


(d) Middle (2000x), 24h storage

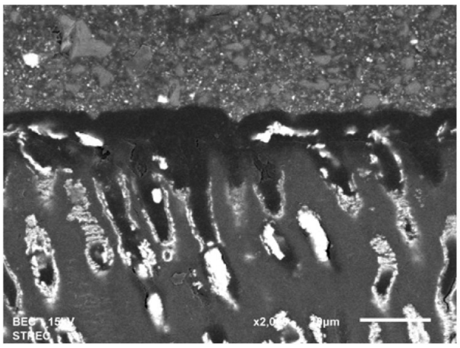
Figure 5 Representative SEM photographs of nanoleakage in ethanol stepwise group (1000x, 2000x) with 24 hours storage at the coronal (a, c) and middle part (b,d)



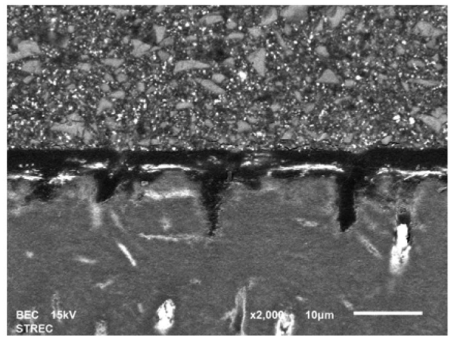
(a) Coronal (1000x), Thermocycling



(b) Middle (1000x), Thermocycling

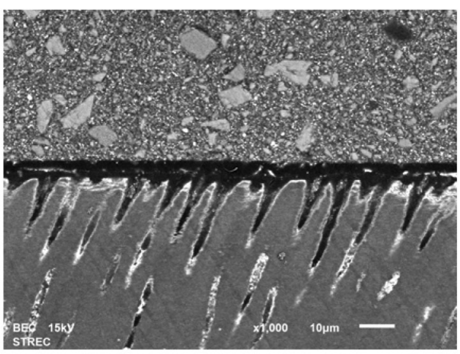


(c) Coronal (2000x), Thermocycling

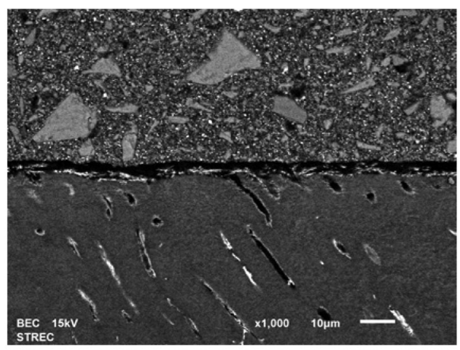


(d) Middle (2000x), Thermocycling

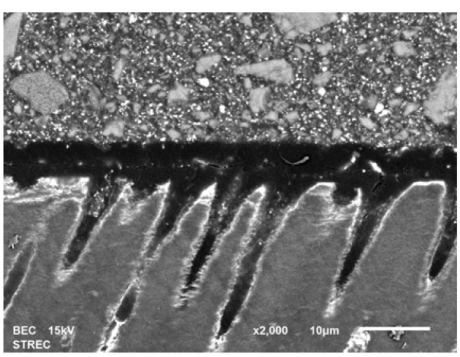
Figure 6 Representative SEM photographs of nanoleakage in control group (1000x, 2000x) with 10,000 cycles of thermocycling at the coronal (a, c) and middle part (b, d).



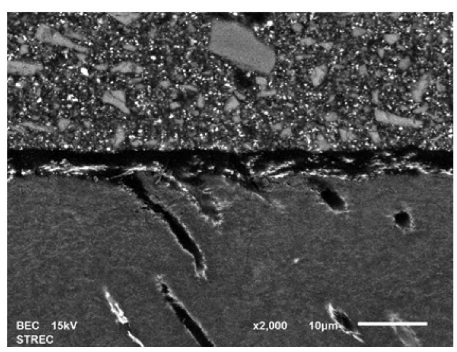
(a) Coronal (1000x), Thermocycling



(b) Middle (1000x), Thermocycling

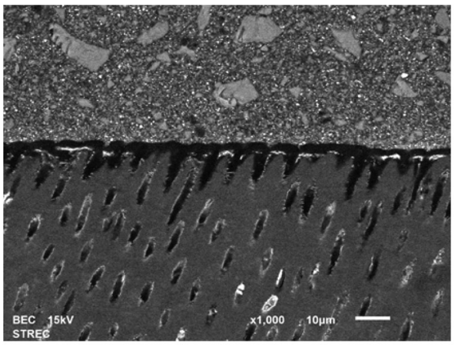


(c) Coronal (2000x), Thermocycling

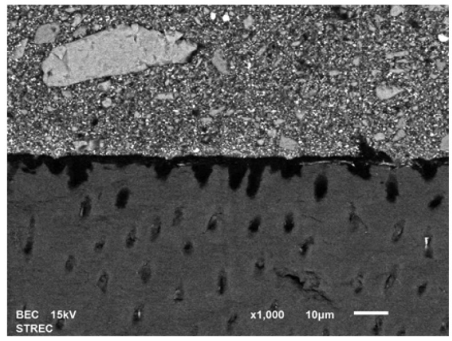


(d) Middle (2000x), Thermocycling

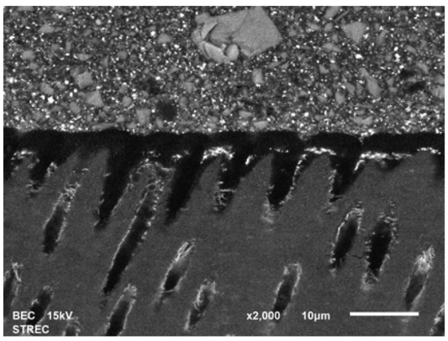
Figure 7 Representative SEM photographs of nanoleakage in ethanol 70% group (1000x, 2000x) with 10,000 cycles of thermocycling at the coronal (a, c) and middle part (b, d).



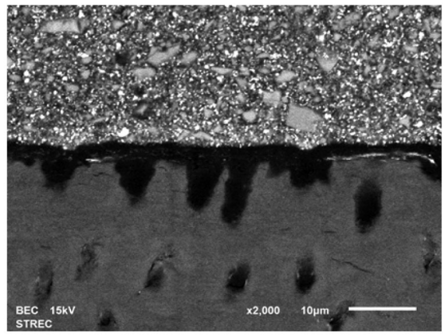
(a) Coronal (1000x), Thermocycling



(b) Middle (1000x), Thermocycling

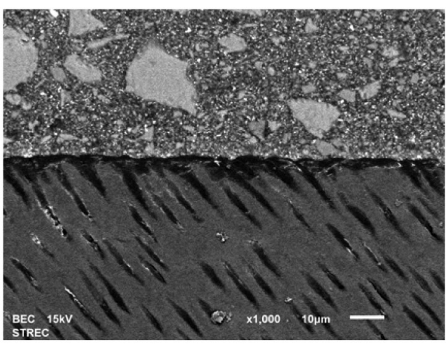


(c) Coronal (2000x), Thermocycling

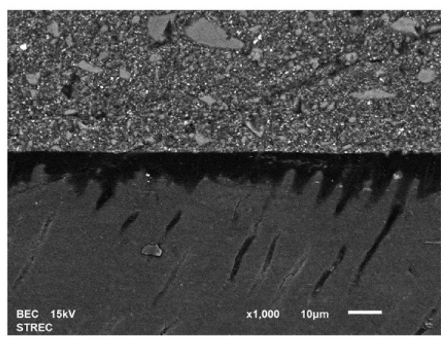


(d) Middle (2000x), Thermocycling

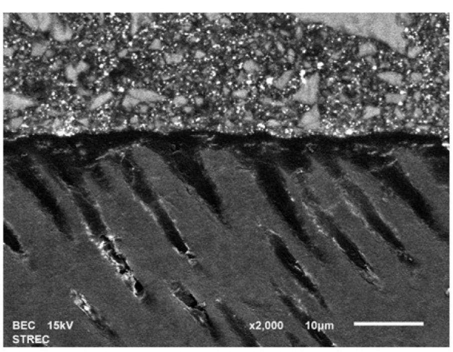
Figure 8 Representative SEM photographs of nanoleakage in ethanol 100% group (1000x, 2000x) with 10,000 cycles of thermocycling at the coronal (a, c) and middle part (b, d)



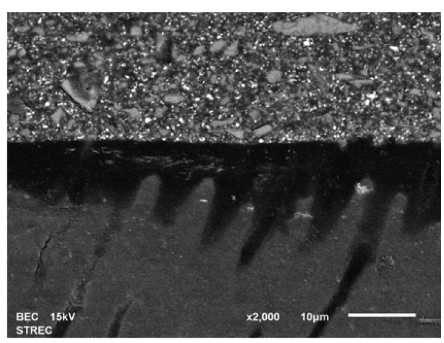
(a) Coronal (1000x), Thermocycling



(b) Middle (1000x), Thermocycling



(c) Coronal (2000x), Thermocycling



(d) Middle (2000x), Thermocycling

Figure 9 Representative SEM photographs of nanoleakage in stepwise ethanol group (1000x, 2000x) with 10,000 cycles of thermocycling at the coronal (a, c) and middle part (b, d).

Discussion

In our present study, various techniques of application of different concentrations of ethanol such as ethanol 70%, ethanol 100% and stepwise ethanol technique as the ethanol-wet bonding technique were performed to eliminate the remained water in post space before bonding procedure. The goal of ethanol-wet bonding is an attempt to keep the moisture in collagen matrix and subsequently to avoid collapsing of the dentin matrix. In addition, the ethanol saturated dentin allows better infiltration of hydrophobic resin and improves the durability of resin since it has solubility parameter close to the co-monomer [14,15].

From the result of our study, there were no significant difference in push out bond strength between 70% ethanol application and control. Since the 70% ethanol composed of 30% of water, thus water was not completely removed from acid-etched dentin. It could be supposed that the 70% ethanol in 60 s could not create the ethanol saturated dentin, which allowed better infiltration of hydrophobic resin and replaced the water within the collagen network of root canal dentin and dentinal tubules. Moreover, the pattern of failure mode with 24 hours storage in control group and 70% ethanol group were almost the same as adhesive failure. Similarly, the pattern of failure mode with 10,000 cycles of thermocycling in control and ethanol 70% group showed in 100% of adhesive failure, regardless of root canal dentin regions. The SEM images from the nanoleakage evaluation of bonded interface in control group with 24 hours storage and thermocycling group (Figure 2 and 6) demonstrated a silver nitrate staining within the adhesive layers. Conversely, the SEM images in ethanol 70% group with 24 hours storage and thermocycling group showed a silver nitrate staining in the bottom of hybrid layers.

Furthermore, the push-out bond strength in 100% ethanol and stepwise ethanol group showed higher the bond strength values than control and 70% ethanol groups. In the stepwise ethanol technique, series of increasing ethanol concentrations (50%, 70%, 80%, 95%) was used for 30 seconds in each solution. Then, 100% ethanol was applied for 30 seconds with 3 times. This ethanol dehydration process is called "full chemical dehydration protocol [16]. Generally, this technique is derived from the tissue embedding technique in which hydrated organic tissues are chemically dehydrated with ethanol by stepwise immersion in and ascending series of solvents.

Conversely, this technique is time-consuming and difficult to perform properly in clinical practice. Previous study, using the different ethanol protocols including 100% ethanol (simplified) application, illustrated that only 100% ethanol was enough to achieve the same result as the stepwise technique [15]. Therefore, all ethanol wet-bonding techniques; 100% ethanol and stepwise technique, provided the similar dentin bond strength [17]. The ethanol-wet bonding technique, used the 100% ethanol for 60 seconds, can replace the residual water in acid-etched dentin, maintain the collagen fibrils, and promote the infiltration of resin monomer [18]. Accordingly, Hosaka et al. observed the use of 100% ethanol application for 60 seconds on dentin surface. It was found that the widening interfibrillar spaces in dentinal hybrid layers was illustrated in TEM observation [14].

Ethanol treatment of acid-etched dentin leads to shrinkage the diameter of collagen fibril and increases in the interfibrillar volume [19]. After stepwise ethanol technique, the volume of the interfibrillar area of collagen network increased by more than 80% [10]. The larger interfibrillar spaces enhances better the penetration of adhesive monomers [20,21] and helps to better encapsulated the exposed collagen fibrils in demineralized dentin [19]. Moreover, the ethanol-saturated dentin produced lower contact angle than water-saturated dentin due to good wettability [22]. Therefore, in our study, the push out bond strength in stepwise ethanol group and 100% ethanol group produced higher bond strength than the control group and 70% ethanol group in both of 24-hour storage and thermocycling group. For this reason, the pattern of failure mode with 24-hour storage in 100% and stepwise group showed decrease in adhesive failure. Similarly, the pattern of failure mode in stepwise technique with 10,000 thermocycling also showed decrease of adhesive failure. Accordingly, the pattern of failure mode was consistent with the result of push-out bond strength. The adhesive failure was mostly found in lower bond strength value. This lower bond strength value was mainly due to incompatibility between water in acid-etch dentin and hydrophobic adhesive [23]. It could be suggested that the propagation of the initial defects at the adhesive-dentin interface resulted in increase of this failure mode pattern.

Regard to the root canal regions, the middle part of root canal dentin in 100% ethanol group and stepwise ethanol group with 24 hour storage showed higher push-out bond strength higher than the coronal part of root canal dentin region. The velocity of polymerization might affect the flow capacity of resin composite, so that the reduced gap widths developed in a resin composite with the lower velocity. The lower velocity of polymerization resulted in better adaptation of the restoration and less internal stresses in resin composite. When light-curing resin composite, a gel stage existed only for a moment, there may not be enough time for resin composite to flow. Therefore, light-curing composite found higher magnitudes of the internal stresses in resin composite than the self-curing composite [24].

The application of a commercially adhesive (ExciTE® F DSC) to root canal dentin with the ethanol application can be used to reduce the interfacial nanoleakage of resin-dentin bonding interface. The location of silver nitrate particles deposits indicates the areas of adhesive layer or hybrid layer where solvent or water remained after the evaporation [25,26]. In our study, the silver particles deposited along the bottom of hybrid layer of 70% ethanol group and 100% ethanol group, regardless of storage time. It might be due to incomplete resin infiltration into acid-etched dentin [27,28]. Moreover, the silver particle also deposited within and along the adhesive layer. The porous formation within the hybrid layer due to the compatibility of water and adhesive could be the reason for the extensive silver uptake. The SEM images (Figure 3-4 and 7-8) demonstrated silver nitrate staining in the bottom of hybrid layers. The adhesives penetration into total demineralized depth of the acid-etched dentin was limited, creating the nanoleakage formation [29].

The nanoleakage evaluation of the bonded interface with stepwise ethanol group in 10,000 cycles of thermocycling showed no silver nitrate uptake. It was similar with that in 24 hours storage. It could be explained that the better resin infiltration and encapsulation of acid-etched dentin by adhesive after the ethanol treatment. Therefore, the ethanol-wet bonding with stepwise technique has influenced the quality of the hybrid layer in according to the figure 5 and 9.

Since the chemical composition of ExciTE® F DSC contains hydroxyethyl methacrylate (HEMA), the water could be trapped in the acid-etched dentin. Moreover, HEMA can reduce the water evaporation, forming porous anionic hydrogels through copolymerization with HEMA. Therefore, the remnant of water may result in the area of incomplete polymerization in the hybrid layer. These areas may permit water permeation, accelerating water sorption and extraction of unpolymerized or degraded monomers. Subsequently, this phenomenon affects the durability of bonding [30].

In our study, the ethanol-wet bonding technique with 100% ethanol and stepwise-ethanol application with dual-cured etch-and-rinse adhesive improved the bond strength of resin composite core material to root canal dentin, compared to the ethanol wet bonding after 10,000 cycles of thermocycling. On the other hand, the nanoleakage formation with dual-cured etch-and-rinse adhesive using stepwise-ethanol application showed no silver penetration after 10,000 cycles of thermocycling, when compared to water-wet bonding, 70% ethanol and 100% ethanol.

Regarding clinical implication, the stepwise application of ethanol-wet bonding with dual-cured etch-and-rinse adhesive improved the bonding and bond durability of resin core material to root canal dentin.

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