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Effects of light curing modes and ethanol-wet bonding on dentin bonding properties*

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Abstract: Objective: This study explored the effects of different light curing modes and ethanol-wet bonding on dentin bonding strength and durability. Methods: A total of 54 molars were randomly divided into three groups: Single Bond 2, Gluma Comfort Bond, and N-Bond. Based on the three light-curing modes and presence or absence of ethanol pretreatment, the samples were assigned to six subgroups: high-light mode, ethanol pretreatment+high-light mode, soft-start mode, ethanol pretreatment+soft-start mode, standard mode, and ethanol pretreatment+standard mode. All samples were bonded with resin based on the experimental groups. After 24 h and 6 months of water storage, a universal testing machine was used to measure microtensile bond strength. Scanning electron microscopy (SEM) was applied to observe mixed layer morphology. Results: The 24-h and 6-month microtensile bond strengths of the ethanol pretreatment groups were significantly higher than those of the non-ethanol pretreatment groups at the same light modes (P<0.05). With or without ethanol pretreatment, the microtensile bond strengths of the high-light modes were significantly lower than those of the soft-start modes and standard modes (P<0.05). The microtensile bond strengths of samples from the 6-month water storage group significantly decreased compared with those of samples from the 24-h water storage group (P<0.05). The soft-start groups and standard groups formed better mixed layers than the high-light mode groups, whereas the ethanol pretreatment groups formed more uniform mixed layers than those without ethanol pretreatment. Conclusions: Ethanol-wet bonding technique, soft-start, and standard modes could improve dentin bonding properties.

Key words: Light curing mode, Soft-start, Ethanol-wet bonding, Scanning electron microscopy (SEM), Bonding strength

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1 Introduction

With continuing in-depth research on the adhesive properties and the increase in people's aesthetic requirements, improving the quality and durability of the bonding interface has become an urgent problem in dentin bonding (Sadek *et al*., 2010; Guimarães *et al*., 2012; Li *et al*., 2012). Although the two-step total-etch technique can achieve high bond strength, there are problems of high technical sensitivity, uncontrollable interface moisture and poor durability. Since the dentin bonding strength is generated after the formation of micro-mechanical retention by the resin monomer in the etched collagen fiber network, the quality of the hybrid layer (Urapepon, 2014) is directly related to the strength and durability, and therefore a uniform and dense hybrid layer can obtain better bonding effect (Marshall *et al*., 2010). The moisture content and the cured extent of the adhesive resin on the dentin surface will affect the quality of

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the mixed layer, and further study of these factors will contribute to the ability to form a good hybrid layer, thus contributing to achieving an ideal bonding effect.

Introduced in the early 1990s, the water-wet bonding technique by Pashley *et al*. (2007) is a method of keeping dentin moist to prevent the collapse of the collagen fibers after etching, which will help the resin monomers penetrate the collagen fiber network. However, water-wet bonding has difficulty in controlling moisture and in standardization, and excessive moisture in the bonding interface often leads to hydrophobic resin monomers not completely penetrating, which results in the negative phenomenon of affecting dentin bonding properties such as incomplete polymerization, micro-phase separation, and nanoleakage (Tay and Pashley, 2003; Cunha *et al*., 2007). Therefore Pashley *et al*. (2007) proposed an ethanol-wet bonding technique where ethanol displacing water in dentin not only can reduce the moisture content of the dentin surface, but also can make it easier for adhesive resin to penetrate the dentin collagen fiber network, thereby facilitating the formation of a dense and homogeneous mixture of a hydrophobic layer to increase its bond strength and durability.

In addition, the resin binder is a highly flowable composite resin, and its main drawback is polymerization shrinkage during light curing (Osiewicz *et al*., 2015). If shrinkage stress during the polymerization process is greater than the adhesive force in the bonding interface, it will generate gaps in the bonding interface and the material inside. Thus, the polymerization shrinkage of a composite resin has a lot of negative effects on clinical applications, such as microleakage, edge color, fracture of teeth or restorative materials, post-operative sensitivity, and secondary caries (Bouschlicher *et al*., 2000; Marghalani, 2014). To facilitate clinical operation and shorten treatment time, high-powered light-emitting diode (LED) light units came into being. Although high intensity irradiation can improve the conversion of the resin monomer and enhance the mechanical properties of the material, the shrinkage stress and elastic modulus of the resin matrix are increased because of the shortening of the time to reach the gel point of the composite resin (Suh *et al*., 1999), which leads to reduced adhesion properties. Hardan *et al*. (2009) proposed the soft-start light curing mode where the initial light intensity has a lower irradiation, which can make the resin flow sufficiently to compensate for the volume shrinkage generated by the polymerization reaction, and then a high-light intensity is used in irradiation to improve the conversion rate of the resin monomer to a maximum extent (Malhotra and Kundabala, 2010). Thus light intensity and light curing modes that directly affect polymerization reaction and the degree of curing, also affect the resin monomer penetrating the dentin collagen fiber network in forming the mixed layer, thereby influencing the dentin bond performance.

In this study, the ethanol-wet bonding technique and light curing modes will apply to the fifth generation total-etch adhesive systems to explore their impact on dentin bonding strength and durability. There were three hypotheses as follows: (1) ethanol would not affect the performance of dentin bonding; (2) different light curing modes would not have different effects on dentin bonding properties; (3) the impacts of three adhesives on bonding properties would exhibit no difference.

2 Materials and methods

2.1 Grouping

A total of 54 third molars without caries were collected. Informed consent was obtained from the donors of the teeth and approved by the Ethics Committee of Jilin University (China). Under running water, a low-speed IsoMet saw (MTI Ltd., USA) was used to cut enamel from the occlusal surface of the molar to expose shallow dentin. To simulate the clinical dentin smear layer's interface, the dentin surface was polished for 60 s using 600-grit SiC paper under running water. All samples were randomly divided into the following three groups based on different adhesives, namely, Single Bond 2 (3M ESPE Ltd., St. Paul, MN, USA), Gluma Comfort Bond (Heraeus Kulze Ltd., Hanau, Germany), and Tetric® N-Bond (Ivoclar Vivadent AG Ltd., Schaan, Liechtenstein).

Based on the three different light curing modes of Dr.'s Light Curing Light (Good Doctors Company, Korea) and with or without ethanol pretreatment, the samples were assigned to six subgroups: Group A, high-light mode; Group B, ethanol pretreatment+

high-light mode; Group C, soft-start mode; Group D, ethanol pretreatment+soft-start mode; Group E, standard mode; Group F, ethanol pretreatment+ standard mode. Light modes and exposure time are shown in Table 1.

Table 1 Instructions for light mode

Light mode	Time (s)	Profile
High-light	10	Continuous energy output power of 1000 mW/cm² for 10 s
Soft-start	10	Output power is raised gradually from 400 to 1200 mW/cm² in 5 s, and power remains at 1000 mW/cm² during the next 5 s
Standard	20	Continuous energy output power of 500 mW/cm² for 20 s

Light intensity as is claimed by the manufacturer

2.2 Bonding procedure

The dentin surface was etched for 15 s by 37% (0.37 g/ml) phosphoric acid gel (Scientific Pharmaceuticals Inc., Pomona, CA, USA) and then rinsed for 30 s with distilled water. The cotton ball was placed on the edge of tooth to suck the excess water. An appropriate amount of adhesive was gently smeared on the moist dentin surface for 10 s using a small brush and lightly dried for 5 s. Subsequently, the adhesive surface was irradiated under different light curing modes according to the experimental groups. For the ethanol pretreatment groups, the etched dentin surface was inserted into 99.7% ethanol for 2 min, and excess ethanol was absorbed by a dry cotton ball. After the curing of adhesives, four layers of Z350 A2 resin (3M ESPE, USA) were stacked up to a height of 4 mm. Each layer was cured individually with the corresponding light mode for 20 s. After bonding, all samples were stored in distilled water at 37 °C for 24 h.

2.3 Measurement of specimens for microtensile bond strength

Under running water, a saw was used perpendicular to the bonding interface to cut the tooth with a cross-sectional area of 0.9 mm×0.9 mm. Cylindrical specimens of approximately 6.0 mm in length were obtained. "No-crack" specimens were randomly divided into two groups (n=10 each group). A group of samples was immediately used for the microtensile bond strength test, and the bonding interface area was measured and recorded with a Vernier caliper (0–150, Tricle Brand, Shanghai, China) with an accuracy of 0.01 mm. Another group of samples was stored in double distilled water at 37 °C for 6 months.

The microtensile fixture was placed on a universal testing machine (AG-Xplus 10KN, Shimadzu, Japan) with a pre-load of 5 N, a sensor of 500 N, and a crosshead speed of 1 mm/min until the specimens fractured. The microtensile bond strengths were calculated by the formula: $\sigma_s=F/(b\times d)$, where σ_s is the microtensile bond strength (MPa), F is the fracture tension (N), b is the sample width (mm), and d is the sample thickness (mm). The fracture type of the interfaces was observed by a stereomicroscope (SZX16, Olympus, Japan) at the magnification of 30×. The fracture types were divided into the following four types: (1) interfacial fracture occurring in the interface of dentin and resin; (2) cohesive fracture of dentin occurring within the dentin; (3) cohesive fracture of resin occurring within the resin; and (4) mixed fracture with interfacial fracture and cohesive fracture.

SPSS 17.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis. Two-way analysis of variance (ANOVA) and multiple comparisons with Tukey's test (5%) were used to analyze microtensile results. A Fisher test was performed to analyze the fracture type of the interfaces.

2.4 Observation of specimens using scanning electron microscopy

Following the aforementioned steps, 18 extracted teeth bonded with resin were prepared, and were cut into 4.0 mm×4.0 mm×1.5 mm. An intermediate portion of samples was etched by 37% phosphoric acid gel and then immersed in 5.25% sodium hypochlorite (Fuchen Chemical Reagent Factory, Tianjin, China) for 10 min. After the water rinse, samples were treated using 50.0%, 70.0%, 90.0%, and 99.7% ethanol for 15 min each, and soaked overnight in 99.7% ethanol. The hybrid layer was observed by field emission scanning electron microscopy (SEM; XL30, Japan) with an accelerating voltage of 10 kV.

3 Results

3.1 Microtensile results

Table 2 shows the results of 24-h microtensile bond strengths, and Table 3 shows the results of

6-month microtensile bond strengths. Under the same experimental conditions, the strengths of Single Bond 2 (SB), Gluma Comfort Bond (GB), and Tetric® N-Bond (NB) did not show a significant difference (P>0.05). The 24-h and 6-month microtensile bond strengths of the ethanol pretreatment groups were significantly higher (P<0.05) than those of the non-ethanol pretreatment groups with the same light modes. With or without ethanol pretreatment, the microtensile bond strengths of the high-light modes were significantly lower (P<0.05) than those of the soft-start and standard modes at 24 h and 6 months of water storage. The microtensile bond strengths of the standard modes and soft-start modes showed no statistical difference (P>0.05).

Compared with 24 h, the microtensile bond strength in 6 months of each group was significantly decreased (P<0.05), but the decline in the microtensile bond strengths between the three adhesives showed no significant difference (P>0.05). The strengths of Groups A–F fell by an average of 54.89%, 40.07%, 40.00%, 25.00%, 38.29%, and 24.89%, respectively. Thus, the decline in the ethanol pretreatment groups was obviously lower than that in the non-ethanol pretreatment groups, and the decline in the soft-start modes and standard mode groups was far less than that in the high-light mode groups.

3.2 Fracture type observations

The fracture types of 24-h microtensile specimens are shown in Fig. 1. The fracture types of 6-month microtensile specimens are shown in Fig. 2. Fisher's exact test showed that the fracture types of all groups were mainly interfacial fracture, and cohesive and mixed fractures were rare. We observed no significant differences among the groups (P>0.05).

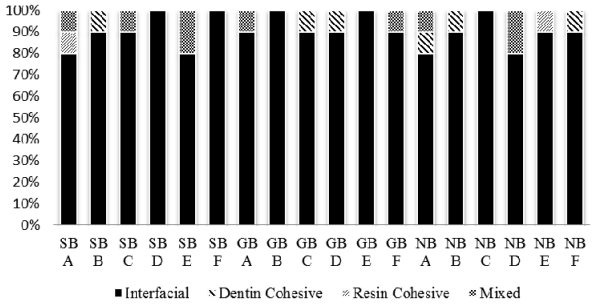

Fig. 1 Fracture types of 24-h microtensile specimens
A: high-light mode; B: ethanol pretreatment+high-light mode; C: soft-start mode; D: ethanol pretreatment+soft-start mode; E: standard mode; F: ethanol pretreatment+standard mode; SB: Single Bond 2; GB: Gluma Comfort Bond; NB: Tetric® N-Bond. The same for the following Figs. 2 and 3

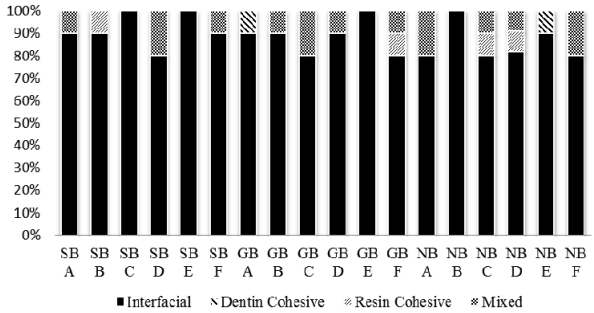

Fig. 2 Fracture types of 6-month microtensile specimens

Table 2 Mean microtensile bond strengths with standard division of 24 h groups (n=10)

Adhesive	Mean microtensile bond strength (MPa)					
	High-light		Soft-start		Standard	
	Null	Ethanol	Null	Ethanol	Null	Ethanol
SB	41.07 ± 3.90^{Aa}	53.44 ± 5.31^{Ab}	51.79 ± 4.41^{Ab}	65.03 ± 4.78^{Ac}	52.06 ± 4.24^{Ab}	67.27 ± 4.44^{Ac}
GB	37.88 ± 3.35^{Aa}	50.03 ± 3.59^{Ab}	49.15 ± 3.91^{Ab}	62.80 ± 4.32^{Ac}	49.19 ± 3.78^{Ab}	63.75 ± 4.33^{Ac}
NB	38.11 ± 3.78^{Aa}	50.32 ± 4.25^{Ab}	49.24 ± 3.63^{Ab}	62.93 ± 4.40^{Ac}	50.50 ± 4.14^{Ab}	64.62 ± 4.79^{Ac}

The same superscripted letters indicate no significant differences (P>0.05). a–c indicate statistically significant differences between different treatment methods (within the same adhesives). A–C indicate statistically significant differences between different adhesives (within the same treatment). SB: Single Bond 2; GB: Gluma Comfort Bond; NB: Tetric® N-Bond. The same for the following Table 3

Table 3 Mean microtensile bond strengths with standard division of 6 months groups (n=10)

Adhesive	Mean microtensile bond strength (MPa)					
	High-light		Soft-start		Standard	
	Null	Ethanol	Null	Ethanol	Null	Ethanol
SB	18.07 ± 3.05^{Aa}	32.07 ± 3.41^{Ab}	31.28 ± 3.52^{Ab}	49.49 ± 4.34^{Ac}	32.06 ± 3.15^{Ab}	51.09 ± 4.22^{Ac}
GB	16.73 ± 3.12^{Aa}	29.40 ± 3.27^{Ab}	29.18 ± 3.04^{Ab}	46.68 ± 4.24^{Ac}	30.72 ± 3.65^{Ab}	47.03 ± 4.62^{Ac}
NB	17.97 ± 2.99^{Aa}	30.70 ± 3.41^{Ab}	29.65 ± 3.52^{Ab}	46.92 ± 3.72^{Ac}	30.85 ± 3.48^{Ab}	48.85 ± 4.59^{Ac}

3.3 Scanning electron microscopy

Fig. 3 shows that the high-light mode groups formed short and uneven resin tags and bubbly uneven mixed layers, whereas the soft-start groups and standard groups formed longer and more uniform resin tags and relatively continuous and uniform mixed layers. The ethanol pretreatment groups formed much longer and more uniform resin tags and more compact and uniform mixed layers than those without ethanol pretreatment. Resin tags and mixed layers formed by the three adhesives were similar.

4 Discussion

In modern bonding technology, the dentin bonding interface frequently has incomplete polymerization, micro-phase separation and nanoleakage, which will adversely affect the performance of the dentin bonding (Cunha *et al*., 2007). Traditional water-wet bonding makes the collapsed collagen fiber network resume a fluffy state, but excess moisture in the bonding interface leads to phase separation and nanoleakage. However, the ethanol-wet bonding

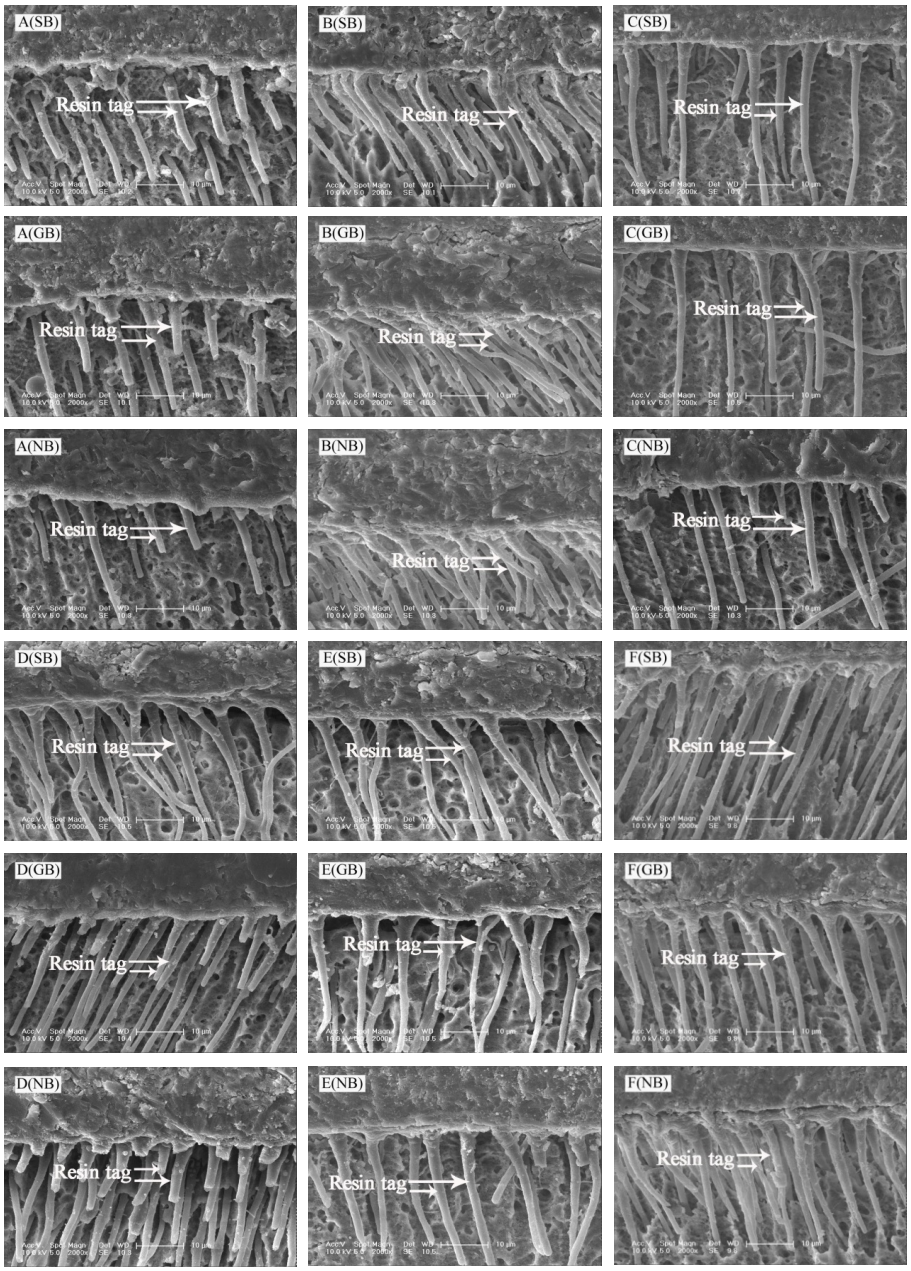

Fig. 3 SEM micrographs of Groups A–F

technique can solve this problem, since ethanol can replace the water in demineralized dentin, occupy gaps between collagen fibers, and reduce dentin hydrophilic, and most of the hydrophobic resin monomers can be soluble in alcohol, so they can better penetrate the dentin collagen fibers and dentinal tubules (Nishitani *et al*., 2006; Li *et al*., 2012; Sartori *et al*., 2015). In addition, ethanol in demineralized dentin can cause transverse shrinkage of collagen fibers that increases gaps between collagen fibers and decreases the collagen matrix hydrophilicity, which provides advantageous locations for subsequent infiltration of hydrophobic resin to form a homogeneous dense hybrid layer in order to obtain good bonding properties (Ahn *et al*., 2015; Yang *et al*., 2016).

Nishitani *et al*. (2006) suggested that the bonding strength of ethanol-wet bonding is higher than that of water-wet bonding. In our experiment, the 24-h and 6-month microtensile bond strengths of the ethanol pretreatment groups are significantly higher ($P<0.05$) than those of the non-ethanol pretreatment groups at the same light modes. With the same processing method, 6-month microtensile bond strength decreases significantly ($P<0.05$), indicating that the degradation of the bonding interface is inevitable in the water environment. The 6-month microtensile test not only shows bonding strength, but also reflects bonding durability. With the same light modes, the decline in microtensile bond strength of the ethanol pretreatment groups is significantly less than that of the non-ethanol pretreatment groups, indicating that ethanol-wet bonding technique will help to improve the durability of the bonding interface. SEM shows that ethanol groups can form more uniform and longer resin tags, and a more uniformly continuous hybrid layer than non-ethanol groups, which is consistent with the microtensile bond strength results, and the dentin bonding property is closely related to the quality of the hybrid layer and the number and length of resin tags. The results deny the first hypothesis, so ethanol-wet bonding technique can form a good dentin bonding interface, thereby significantly improving dentin bonding strength and durability of the total-etch adhesive.

Light curing intensity and pattern can influence the degree of curing of the resin binder, thus affecting its bonding properties (Haenel *et al*., 2015). Fahmy *et al*. (2009) reports that the pulse mode and fast mode have similar performance, so we chose high-light mode, soft-start mode, and normal-light mode instead of pulse mode in this experiment. To explore the effects of light modes on dentin bonding properties clearly, the irradiation time of the three modes is selected to be approximately of the same total light energy (Rueggeberg, 1998).

Light curing resin binder can incur a polymerization shrinkage reaction under the irradiation of certain wavelengths of visible light. This polymerization shrinkage will cause bonding internal stress increases that trigger microcracks in the bonding interface and materials, and these gaps may lead to microleakage and reduction in bond strength within the bonding interface (Giachetti *et al*., 2006). This is the main reason for the failure of repair materials (Mohammad *et al*., 2006). High-light intensity can improve the depth of curing and degree of conversion of the resin monomer to enhance mechanical properties of the composite resin (Nalcaci *et al*., 2005; Alto *et al*., 2006; Filho *et al*., 2008). Since curing speed is proportional to the square root of light intensity (Visvanathan *et al*., 2007), the curing speed of the high-light mode is faster and this can produce a larger polymerization shrinkage and internal stress within the bonding interface. It is not conducive to penetration and stretch of monomer into the dentin collagen fiber network, thus forming shorter resin tags and an asymmetrical mixed layer. In addition, it will make the oxygen inhibition layer thin on the adhesive surface (Kim *et al*., 2006) and make dentinal tubule fluid increasingly penetrate the adhesive surface (Breschi *et al*., 2008), thus affecting its bonding performance.

The soft-start mode is to start with a lower light intensity that extends the flow time of the resin to offset polymerization shrinkage stress, and then irradiate with a higher light intensity to fully cure the resin. Some scholars believe that the soft-start mode cannot reduce polymerization stress and microleakage, and that it has no significant effect on improving marginal adaptation in the bonding interface and degree of conversion of the resin monomer (Bouschlicher *et al*., 2000; Fleming *et al*., 2007). Too high or too low initial intensity cannot achieve a good curing effect (Marghalani, 2014), so the initial light intensity of the soft-start mode in our experiment is

400 mW/cm². Cunha *et al*. (2007) reported that the soft-start mode did not reduce the degree of conversion, but led to lower shrinkage stress and higher bond strength, which demonstrated the correlation with shrinkage stress and bond strength. When the adhesive surface is irradiated with a lower-intensity irradiation, phototropism shrinkage stress generated by the resin monomer is lower (Tseng *et al*., 2007). It is conducive to penetration and stretch of monomer into the dentin collagen fiber network to form longer and thicker resin tags and the dense and uniform mixed layer, and thus its microtensile bond strength will be higher. The experimental results show that with the same pretreatment, whether the water storage is 24 h or 6 months, the microtensile bond strength of the high-light model group is lower than those of the soft-start group and the standard mode group. The SEM shows that high-light mode groups formed short and uneven resin tags and bubbly uneven mixed layers, whereas the soft-start groups and standard groups formed longer and more uniform resin tags and relatively continuous and uniform mixed layers. It is consistent with the above views and denies the second assumption. So, different light curing modes have different effects on dentin bonding properties.

Microtensile results show that, in the same experimental conditions, the strengths of SB, GB, and NB groups at 24 h and 6 months water storage have no significant difference ($P>0.05$), and the declines of the microtensile bond strengths of three adhesives from 24 h to 6 months also show no significant difference. Microtensile bond strengths of different adhesives are different due to the different compositions and resin fillers. Single Bond 2 with the higher monomer concentration can form the thicker mixing layers after evaporation of solvents, and the component of polyalkenoic acid copolymer can enhance chemical combination with structures (Hashimoto *et al*., 2002). Silica nanofillers in Single Bond 2 can not only more easily penetrate the collagen fiber network, but also reduce volume shrinkage during polymerization, which can increase bond strength.

5 Conclusions

Dentin bonding performance is closely related to the quality of the mixed layers, and ethanol pretreatment can significantly improve the performance of total-etch dentin bonding systems and the quality of the mixed layer. Soft-start mode and standard mode can obtain better dentin bonding properties and quality of the mixed layer. The three total-etch adhesives have no significant difference on microtensile results or the quality of the mixed layers.

Compliance with ethics guidelines

Mu-zi LI, Jin-rui WANG, Hong LIU, Xia WANG, Kang GAN, Xiu-ju LIU, De-li NIU, and Xiao-qing SONG declare that they have no conflict of interest.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5). Informed consent was obtained from all patients for being included in the study.

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中文概要

题　目：不同光固化模式和乙醇湿粘接对牙本质粘结性能的影响

目　的：探究不同光固化模式和乙醇湿粘接对牙本质粘结强度及耐久性的影响，为临床应用提供理论指导。

创新点：率先将乙醇湿粘接技术和不同光固化模式有机结合，成功筛选出了提高牙本质粘结性能的有效策略。

方　法：将 Single Bond 2（SB）组、Gluma Comfort Bond（GB）组和 N-Bond（NB）组分别分为 6 个亚组：高光照射模式、乙醇预处理+高光照射模式、软启动照射模式、乙醇预处理+软启动照射模式、标准照射模式和乙醇预处理+标准照射模式。按照分组粘结，水储 24 小时和 6 个月后，应用万能测试机分别检测微拉伸强度，体视显微镜观察微拉伸试件断裂类型和扫描电镜观察混合层形态。

结　论：乙醇湿粘接技术可以改善混合层质量，从而提高其粘结强度和改善粘结耐久性；软启动光照模式和标准光照模式可以形成更好的混合层质量，获得更高的牙本质粘结强度和更好的粘结耐久性。

关键词：光照模式；软启动；乙醇湿粘接；粘结强度；扫描电子显微术