Pre-treatment of radicular dentin by self-etch primer containing chlorhexidine can improve fiber post bond durability

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We evaluated whether the pre-treatment of radicular dentin by ED Primer containing different concentrations of chlorhexidine can improve the bond durability of fiber post to radicular dentin. Experimental ED primers containing different concentrations of chlorhexidine (0%, 0.5% and 1.0%) were prepared. Thirty extracted maxillary anterior teeth were divided into 3 groups, each group corresponding to different chlorhexidine concentrations. Fiber posts were cemented in endodontically treated teeth with experimental ED primers and Panavia F. The bonded teeth were transversally sectioned into six slices and then were processed for thin slice push-out test 24 h later or after 18-months water storage. Eighteen-month storage resulted in significant bond strength reduction of all groups ($p<0.05$). The bond strength reduction of 1.0% group was significantly lower than that of control group and 0.5% group ($p<0.05$). In conclusion, the incorporation of 1.0% chlorhexidine into ED primer can extend the bond longevity of fiber post to radicular dentin.

Keywords: Chlorhexidine, Bond durability, Fiber post, Radicular dentine, Self-etch adhesive

INTRODUCTION

Fiber-reinforced posts have been widely used because of many advantages, such as the similar mechanical characteristics to dentin, biocompatibility, resistance to corrosion, improvement of light transmission and so on. To improve the retention of the composite core to post and tooth, and also the fracture strength of endodontically treated teeth, prefabricated fiber posts are cemented with adhesives and composite resin luting cements. Adhesion between resin and dentin is considered to be a weak point in luting a fiber post[1-3)]. In using resin cements with radicular posts, it is important to maximize the bond strength between the resin and dentin.

Although the immediate bond strength of resin to dentin is quite high, considerable evidences have shown that resin-dentin bond is not durable[4-6)]. There are many possible causes leading to the poor dentin bond durability. One of the most important mechanisms is considered to be the degradation of exposed collagen of the dentin hybrid layers[4,7-9)]. Host derived matrix metalloproteinases (MMPs) were proven to be a key factor in hydrolyzing the exposed collagen[10-15)]. As chlorhexidine possesses desirable MMP-inhibitory properties[16)] and outstanding substantivity to human dentin[17,18)], it has been well studied as a MMP-inhibitor to prevent dentin bond degradation[13,14,19-26)]. Recent studies revealed that cysteine cathepsins are also present in intact and carious dentin which may also contribute to the breakdown of the exposed collagen in dentin hybrid layers[27,28)], and that chlorhexidine can also inhibits the activity of dentin cysteine cathepsins[29)].

Although the effect of chlorhexidine on the bond durability of coronal dentin to resin is well studied, few studies have evaluated the effect of chlorhexidine application on the bond durability of radicular dentin to resin. It has been reported that not only are the MMPs present in coronal dentin, but also present in radicular dentin[30,31)], and that self-etch adhesives can increase the MMPs activity in radicular dentin[32)]. As root canal presents a quite different situation compared with coronal dentin, the application of chlorhexidine in radicular dentin is worth to be studied. The aim of this study is to evaluate the influence of chlorhexidine application on the bond durability of fiber posts to radicular dentin. The first null hypothesis was that the application of chlorhexidine in a self-etch adhesive primer (ED primer, Kuraray, Osaka, Japan) has no adverse effect on the immediate bond strength of fiber posts to radicular dentin. The second null hypothesis was that the application of chlorhexidine could not prevent the bond strength reduction of fiber posts to radicular dentin.

MATERIALS AND METHODS

Preparation of a self-etch primers containing chlorhexidine

A dual-cured resin cement (Panavia F, Kuraray, Osaka, Japan) was tested in this study. Panavia F is used in combination with the proprietary one-step self-etch primer (ED Primer), which include liquid A and liquid B. Different amounts of 20% chlorhexidine digluconate

Color figures can be viewed in the online issue, which is available at J-STAGE.

Received May 16, 2012: Accepted Dec 20, 2012
doi:10.4012/dmj.2012-134 JOI JST.JSTAGE/dmj/2012-134

(Alfa Aesar, Ward Hill, MA, USA) were added directly into ED primer (both liquid A and liquid B) to prepare mixtures containing three different concentrations of chlorhexidine: 0%, 0.5% and 1.0%. The composition of Panavia F and ED primer are seen in Table 1.

Tooth preparation and bonding procedure

Thirty extracted human maxillary anterior teeth were collected. The informed consent was obtained. The study protocol was reviewed and approved by the Ethics Committee for Human Studies, Peking University, Beijing, China. These teeth were stored in 0.9% NaCl containing 0.02% sodium azide at 4ºC for no more than one month.

The crown portion of each tooth was removed using a water-cooled diamond bur below the cementum-enamel junction, and perpendicular to the long axis of the tooth. The roots were endodontically instrumented at a working length of 1 mm from the apex with a #35 master apical file. A step-back technique was used with stainless-steel K-files, and 2% chloramines irrigation. The roots were obturated with gutta-percha (Liuyuan, Dayading Medical Appliance Co.,Ltd, Tianjin, China), and a eugenol-free root-filling material (VitaPex, Morita, Tokyo, Japan). Part of this filling material was then removed with Gates-Glidden drills #2, and the canal wall of each specimen was enlarged with low-speed post drills provided by the post manufacturer, to create a 9 mm deep post space, as measured from the cementum-enamel junction of the tooth. A 1.5 mm diameter conical glass fiber post (LuxaPost, DMG, Hamburg, Germany) was tried-in and then cleaned with alcohol and dried with oil-free air. The largest diameter in the coronal region of the post is 1.5 mm and the diameter of the tip end in the apical region is 0.94 mm. The taper rate of the posts is 0.07, that is, the conical part reduces its diameter 0.07 mm per 1 mm length.

The thirty teeth were randomly allocated to 3 groups, each group corresponding to one of the chlorhexidine concentrations: 0%, 0.5%, and 1.0%. Panavia F was used strictly according to the manufacturers' instructions to lute the posts: before the application of adhesive resin cement, one drop each of Primer liquid A and liquid B were mixed for 5 s and applied to the post space walls with a microbrush for 30 s. Excess primer solution was removed with paper points, and the primer was then gently air dried. For cementation of fiber posts, equal amounts of a dual-polymerized resin luting agent paste base and catalyst were thoroughly mixed and applied to the post space walls with a spiral instrument (Mani Inc, Tochigi, Japan). The posts were then seated to full depth in the prepared spaces using finger pressure, and excess luting agent was removed with a cotton pellet. Subsequently, the resin cement was light polymerized for 40 s with a halogen light-curing unit (LITEX 682, Dentamerica, CA, USA) with an output of 700 mW/cm^2. The bonded teeth were then stored in distilled water at 37°C for 24 h until the specimen preparation. The bonding procedures are seen in Table 1.

Thin slice push-out test

The schema of the experimental procedures of the thin slice push-out test are seen in Fig. 1. The bonded teeth were embedded in acrylic resin. Parallelism between post, canal and resin block was obtained using a parallel meter (see Fig. 1(b)). The acrylic blocks were then transversally sectioned into six slices (about 0.7 mm thick) using a low speed diamond saw (Isomet 1000, Buehler Ltd, NY, USA) under water cooling. The thickness of each slice was individually measured by means of a digital caliper (SH100, SHAHE, Chengdu, China). Both the coronal and apical post diameter were measured by using a toolmaker's microscope (176-901E, Mitutoyo, Kawasaki, Japan). The bonding surface was calculated using the formula of a conical frustum:

$$\pi(R_1+R_2)([R_1-R_2]^2+h^2)^{1/2}$$

where π is the constant 3.14, R_1 is the coronal post radius, R_2 is the apical post radius and h is the thickness

Table 1 The composition of Panavia F and ED primer and the application procedure

Material	Composition	Batch#	Application procedure
Panavia F	Paste A: 10-methacryloyloxydecyl dihydrogen phosphate (MDP), hydrophobic and hydrophilic dimethacrylate, benzoyl peroxide, camphoroquinone, colloidal silica	00250G	Mix equal amounts of ED primer liquids A and B, apply mixture to the post space with a microbrush for 30 s, remove excess with paper points and then gently air-dry. Mix Panavia F paste A and B for 20 s, apply the mixed paste to the post space walls, seat the posts in place, remove excess luting agent, light cure for 40 s with a halogen light-curing unit with an output of 700 mW/cm^2
	Paste B: sodium fluoride, hydrophobic and hydrophilic dimethacrylate, diethanol-*p*-toluidine, T-isopropylic benzenic sodium sulfinate, barium glass, titanium dioxide, colloidal silica	00027H	
ED Primer	Liquid A: 2-hydroxyethyl methacrylate (HEMA), MDP, NM-aminosalicylic acid, diethanol-*p*-toluidine, water	00272A	
	Liquid B: NM-aminosalicylic acid, T-isopropylic benzenic sodium sulfinate, diethanol-*p*-toluidine, water	00147A	

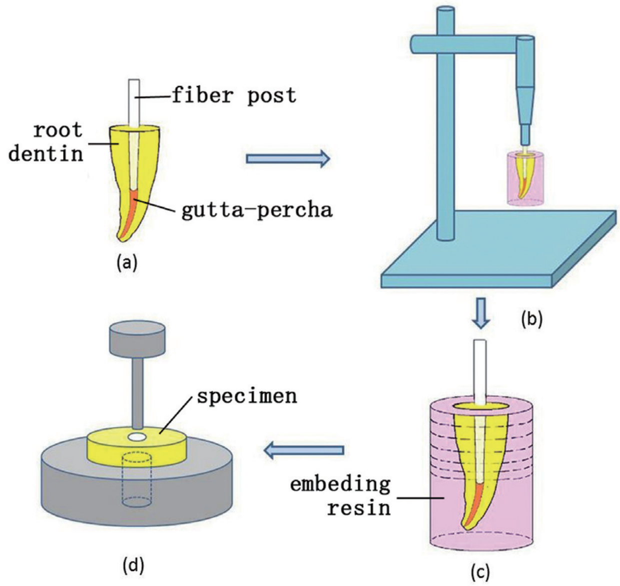

Fig. 1 Schema of the experimental procedures of the thin slice push-out test.

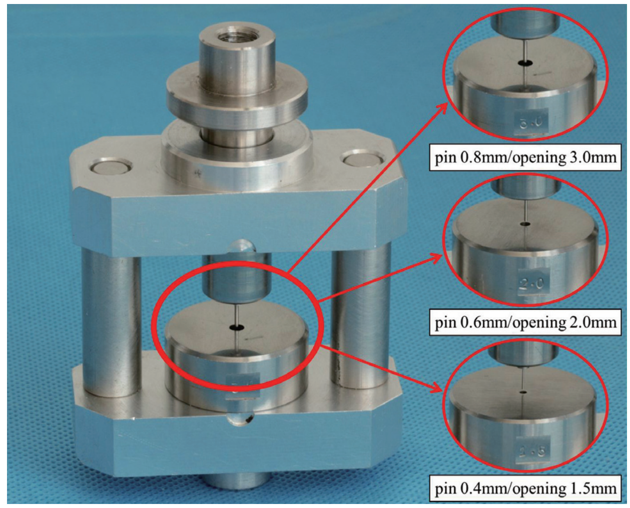

Fig. 2 The loading fixtures for thin slice push-out test.

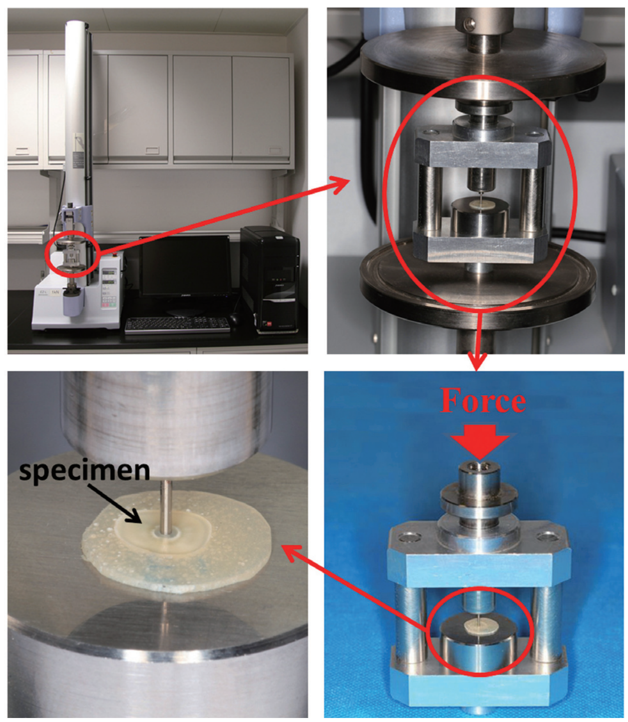

Fig. 3 The thin slice push-out test.

of the slice in mm.

After the slicing process, half of the teeth from each group (five teeth from each group, 6 slices from each tooth. Therefore, in every subgroup, the sample size was 30) were tested 24 h later to measure the immediate bond strength. The other half teeth of each group (n=30) were stored in 0.9% NaCl containing 0.02% sodium azide at 37ºC for 18 months before testing to evaluate the bond durability.

The slices were firmly fixed with cyanoacrylate glue to a loading fixture. A compressive load at a speed of 0.5 mm/min was applied on the apical aspect of the slice *via* a universal testing machine (EZ-L-1kN, Shimadzu, Tokyo, Japan). The loading force was applied in an apical-coronal direction, in order to move the post towards the larger part of the root slice. The punch pin was loaded within the fiber post. With regards to the tapered design of the post, three different sizes of punch pins as well as three different openings were used for the push-out testing. The diameter of the punch pin was 0.8 mm and the diameter of the opening 3.0 mm for the two coronal slices, 0.6 mm and 2.0 mm for the two middle slices, and 0.4 and 1.5 mm for the two apical slices. It was thus guaranteed that the overlaying root dentin was sufficiently supported during the loading process. The loading fixtures for the thin slice push-out test are seen in Fig. 2. The thin slice push-out test are seen in Fig. 3.

In order to express the bond strength in MPa, the load at failure recorded in Newtons was divided by the area of the bonded interface.

Debond pathway determination

The failure mode of each debonded specimen was assessed under a stereomicroscope (Olympus 220670; Tokyo, Japan) with 40× magnification to record the failure modes. The fracture modes were classified as follows: (1) cohesive failures in the dentin; (2) cohesive failures in the post; (3) adhesive failures in the joint between dentin and cement; (4) adhesive failures in the joint between post and cement; and (5) mixed failures, when two or more failure modes were present within the same specimen. The pictures of the failure modes are seen in Fig. 4.

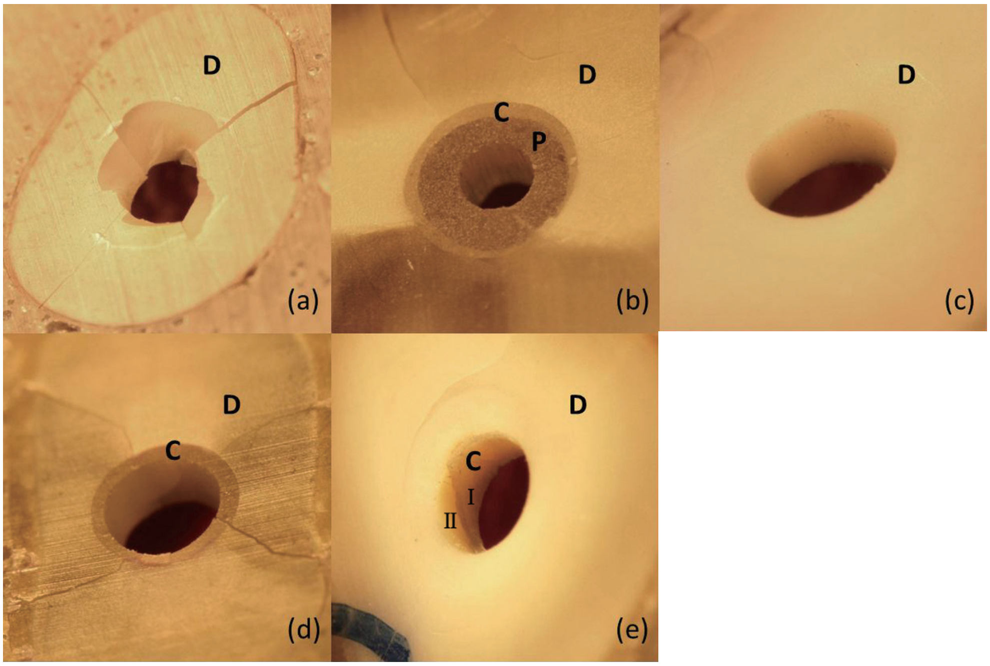

Fig. 4 The failure mode of debonded specimens.
D, dentin; C, cement; P, post.
(a) cohesive failures in the dentin: the post and cement and part of root dentin was pushed out and we can identify the cracks in the root dentin due to the loading force; (b) cohesive failures in the post: the debonded surfaces were totally within the posts, with the bond surface between dentin and cement and the bond surface between cement and post were intact; (c) adhesive failures in the joint between dentin and cement: the post and cement was completely pushed out, leaving the root dentin intact, we can see the smooth surface of the root dentin; (d) adhesive failures in the joint between post and cement: the post was completely pushed out, leaving the cement and root dentin intact. We can see the smooth surface of the cement; and (e) mixed failures in the joint between dentin and cement and in the joint between post and cement: the post and part of the cement was pushed out, leaving residual cement on the intact dentin. We can see two surfaces: I, the debonded surface between post and cement; II, the debonded surface between cement and dentin.

Statistical analysis

A one-way analysis of variance (ANOVA) and post hoc multiple comparisons was used to compare the effect of dentine treatment (control *vs*. chlorhexidine) before or after water storage, and the independent *t* test was used to compare the effect of water storage time (24 h *vs*. 18 mos) on each group. A one-way analysis of variance (ANOVA) and post hoc multiple comparisons was used to compare the bond strength from different root regions (coronal region, middle region or apical region). An exact chi-square test was used to analyze the distribution trend of 3 groups at the 24 h testing period, and to compare the differences of failure mode distribution before and after water storage. Statistical significance was pre-set at α=0.05. The statistical unit was slices, not teeth.

RESULTS

Push-out strengthes

The results of the push-out strength measurements are represented in Table 2. There is no significant difference of immediate bond strength between the control group and any of the experimental groups (p>0.05). After 18 months water storage, the bond strength of all groups decreased significantly (57.0%, 45.5% and 30.1% respectively) compared with respective immediate bond strength (p<0.05). The bond strength of 1.0% Chlorhexidine (18 mos) group was significantly higher than that of Control (18 mos) group and 0.5% Chlorhexidine (18 mos) group (p<0.05). There is no significant difference of bond strength between the Control (18 mos) group and 0.5% Chlorhexidine (18 mos) group (p>0.05).

The results of the push-out strength from different radicular dentine regions are represented in Table 3. In all groups, the "coronal region" had higher push-out strength than the "middle region" and the "apical region". In the Control (24 h), Control (18 mos), 0.5% Chlorhexidine (18 mos) and 1.0% Chlorhexidine (18 mos) groups, the differences have no statistical

Table 2 Thin slice push-out test results (MPa)

Group	Number of specimens tested	Immediate bond strength	After 18 months aging
Control	60	16.08 (7.22)^{a}	6.91(2.83)^{b}
Chlorhexidine (0.5%)	60	15.70 (6.82)^{a}	8.55(3.41)^{b}
Chlorhexidine (1.0%)	60	16.37 (7.15)^{a}	11.44(4.51)^{c}

Values identified by different letters are significantly different (p<0.05).

Table 3 Thin slice push-out test results of different radicular dentine regions (MPa)

Groups	Coronal region	Middle region	Apical region
Control (24 h)	17.56 (8.05)^{1,a}	13.92 (6.15)^{1,b}	16.76 (7.56)^{1,c}
Control (18 mos)	7.52 (2.75)^{2,d}	6.27 (1.99)^{2,f}	6.95 (3.66)^{2,g}
0.5% Chlorhexidine (24 h)	20.21 (8.84)^{3,a}	13.58 (4.40)^{4,b}	13.30 (4.31)^{4,c}
0.5% Chlorhexidine (18 mos)	9.80 (4.78)^{5,d}	8.08 (2.32)^{5,f}	7.76 (2.56)^{5,g}
1.0% Chlorhexidine (24 h)	21.93 (7.87)^{6,a}	14.15 (6.05)^{7,b}	13.05 (3.78)^{7,c}
1.0% Chlorhexidine (18 mos)	13.48 (5.57)^{8,e}	10.47 (2.33)^{8,f}	10.38 (4.71)^{8,g}

Values identified by different numbers and letters are significantly different (p<0.05). The numbers illustrate the difference of bond strength among different radicular dentine regions in every subgroup. The letters illustrate the difference of bond strength among different subgroups when compared regionally.

Table 4 Distribution of the failure mode

Control/ Experimental group	Failure mode									
	Cohesive failures in the dentin		Cohesive failures in the post		Adhesive failures in the joint between dentin and cement		Adhesive failures in the joint between post and cement		Mixed failures	
	24 h	18 mos	24 h	18 mos	24 h	18 mos	24 h	18 mos	24 h	18 mos
Control	0/30	0/30	4/30	0/30	11/30^{a}	18/30^{b}	6/30	2/30	9/30	10/30
Chlorhexidine (0.5%)	3/30	0/30	0/30	0/30	10/30^{c}	11/30^{c}	9/30	5/30	8/30	14/30
Chlorhexidine (1.0%)	1/30	0/30	1/30	0/30	8/30^{d}	12/30^{d}	4/30	2/30	16/30	16/30
Total	4/90	0/90	5/90	0/90	29/90^{e}	41/90^{f}	19/90^{g}	9/90^{h}	33/90	40/90

xx/XX: xx=numbers of sticks tested reporting the indicate failure mode; XX= total sticks tested. Values identified by different letters are significantly different (p<0.05).

significance (p>0.05). In the 0.5% Chlorhexidine (24 h) and 1.0% Chlorhexidine (24 h) groups, the differences have statistical significance (p<0.05). When analyzed regionally, there is no significant difference of immediate bond strength between the control group and any of the experimental groups in all radicular dentine regions (p>0.05). After 18 months water storage, the bond strength of 1.0% group was higher than that of control group and 0.5% group in all radicular dentine regions. But only in the coronal region, the difference had statistical significance (p<0.05)

Distribution of the failure mode

Table 4 summarizes the distribution of the failure modes. At the 24 h testing period, most failure modes were adhesive failures between dentin and cement or adhesive failures between post and cement or mixed failures of both. There were few cohesive failures in the dentin or in the post. The trend test showed that there are no differences of distribution trend between the control group and any of the experimental groups (p>0.05).

After 18-month of aging time, most failure modes were adhesive failures between dentin and cement or mixed failures. When counted together, the adhesive failures between dentin and cement increased, along with a decrease in adhesive failures between post and cement ($p<0.05$). When analyzed separately, the adhesive failures between dentin and cement in the control group increased significantly ($p<0.05$), while there is no statistical difference before and after water storage in the two experimental groups ($p>0.05$). Most mixed failures were the combination of adhesive failures between dentin and cement or adhesive failures between post and cement.

DISCUSSION

Since there was no significant difference of immediate bond strength between the control group and experimental groups, the first hypothesis is accepted, indicating that chlorhexidine has no adverse effect on the immediate bond strength of fiber posts to radicular dentin. After 18-month storage, the bond strength of 1.0% group was significantly higher than that of control group, showing that the incorporation of chlorhexidine into ED primer of Panavia F can extend the bond longevity of fiber posts to radicular dentin. Therefore, the second hypothesis has to be rejected. However, even in the 1.0% group, the bond strength reduction after water storage is still significant (about 30%), indicating that chlorhexidine can not totally preserve the bond strength of fiber posts to radicular dentin.

There are mainly three methods to evaluate the bond strength of fiber post to radicular dentin: push-out test, thin slice push-out test and microtensile test. Push-out test has benefit of more closely simulating the clinical condition. However it was suggested that a highly non-uniform stress may be developed at the adhesive interface when the push-out test is performed on the whole post or on thick root sections[33,34]. Compared with push-out test, the thin slice push-out test permits a more uniform stress distribution along the bonded interface[35,36]. Microtensile technique was reported to have great number of premature failures and high standard deviation values[36]. Therefore, the thin slice push-out test appeared to be more dependable than the microtensile technique and Push-out test when measuring the bond strength of luted fiber posts.

Long-term water storage and fatigue resistance test, including thermal cycling and cyclic loading test, are the conditions most often used to test the durability of resin bonds. All these tests are considered to be clinically relevant aging parameters. The thermal cycling and cyclic loading test were used to simulate the clinical condition more closely. However, this study was designed to evaluate the inhibitory effect of chlorhexidine on dentin MMPs to improve the bond durability of fiber post to radicular dentin. Fatigue resistance test, especially the thermal cycling test, might interference the MMP activity, adding influence factors to the results. Therefore, this study chose long-term water storage to test the bond durability of fiber post to radicular dentin.

With regards to the tapered design of the post, three different sizes of punch pins (0.8 mm, 0.6 mm and 0.4 mm) as well as three different openings (3.0 mm, 2.0 mm and 1.5 mm) were used for the push-out testing. It can guarantee the overlaying root dentin be sufficiently supported during the loading process. However, this could also affect the results of bond strength. Therefore, in this study, we calculated the bond strength according to different regions of radicular dentine. The results illustrated that the "coronal region" had higher push-out strength than the "middle region" and the "apical region" in all groups. This result was in accordance with many other previous studies[37-40]. This might be because the light intensity at the deep level of the root canal may be insufficient to induce proper polymerization of the adhesive cement. When analyzed regionally, chlorhexidine had no adverse effect on the immediate bond strength in all radicular dentine regions; after 18 months water storage, the bond strength of 1.0% group was higher than that of control group and 0.5% group in all radicular dentine regions, although the differences in the "middle region" and "apical region" had no statistical significance ($p<0.05$). These results were similar to the results analyzed totally. Therefore, the effect of chlorhexidine on the radicular dentin has no regionally difference.

The results of this study showed that the incorporation of chlorhexidine (0.5% or 1.0%) into ED primer of Panavia F has no negative effect on the immediate bond strength of fiber posts to radicular dentin. These results are similar to Hiraishi *et al.*[41]. Hiraishi *et al.* reported to incorporate chlorhexidine as an antibacterial agent into the ED primer 2.0 of Panavia F 2.0 and found that the 1% chlorhexidine-containing primer had no adverse effect on the immediate bond strength of resin to coronal dentin[41]. They also reported that the mild acidity and the demineralising effect of the ED primer were not affected by the incorporation of chlorhexidine as long as the concentration of chlorhexidine is no higher than 1.0%[41]. All these results revealed the feasibility of incorporating chlorhexidine into the self-etch primer of luting cements.

Leitune *et al.*[42] recently reported to apply chlorhexidine after dentin phosphoric acid etch to evaluate the influence of chlorhexidine application at immediate and long-term bond strength of fiber post cemented to root dentin. After 6 months water aging, they found that chlorhexidine application did not effectively arrest bond strength reduction of fiber posts to root dentin. They hypothesized that 6 months of storage may not have been enough to show an effect of chlorhexidine in preventing the collagenolytic effect of MMPs. Cecchin *et al.*[3,43] also applied chlorhexidine in an etch-and-rinse bonding systems and stored the specimens in water for 12 months. They concluded that the use of chlorhexidine pretreatment could preserve the bond strength of fiber post to radicular dentin after 12 months storage. Although there were studies on the use of chlorhexidine in etch-and-rinse adhesives[3,42,43] and there was a study to evaluate the effect of chlorhexidine application in self-etch adhesive on the immediate bond strength of resin to radicular dentin[41], very little information is available on the application of chlorhexidine in conjunction with self-etch adhesives to improve the bond durability of resin to radicular dentin. Compared with etch-and-rinse bonding systems, self-etch bonding systems do not require rinsing after etching, and hence are more user-friendly and have lower technique sensitivity. Therefore, it is worthy to apply chlorhexidine in self-etch bonding systems. In one of our previous studies, chlorhexidine was applied in a self-etch adhesive (Clearfil SE Bond, Kuraray, Osaka, Japan) to improve the bond durability of resin to coronal dentin[26]. To simplify bonding procedure, chlorhexidine was incorporated directly into the primer of SE Bond. The results illustrated that when incorporated in the primer of Clearfil SE Bond, chorhexidine can preserve dentin bond for at least one year. In this study, chlorhexidine was also incorporated into the ED primer of Panavia F. After 18-months water storage, the effect of chlorhexidine in preventing the bond degradation of fiber posts to radicular dentin was identified.

In this study, after water storage, adhesive failures between dentin and cement increased, along with a decrease in adhesive failures between post and cement. This phenomenon is in accordance with previous studies[1-3] and illustrated that the adhesion between resin and dentin is a weak point and a determinant of the fiber posts bond durability. The adhesive failures between dentin and cement in the control group increased much more than that in the two experimental groups, which also proved the positive effect of chlorhexidine on the fiber posts bond durability.

In this study, even in the 1.0% group, a significant bond strength reduction (about 30%) can also be observed, although it was significantly lower than that of the control group and 0.5% group (about 57% and 46% respectively). This might be due to the much more complexity of fiber post bonding, which involves two different bond interfaces: dentin/cement and cement/post. The root canal presents a different and more challenging environment compared with coronal dentin. There is low light penetration into the root canal during polymerization, and a low degree of conversion of formed polymer[44,45]. The polymerization contraction could also affect the cement/dentin interface according to cavity configuration[46]. The significant reduction of bond strength in the 1.0% group demonstrated that apart from the MMP-hydrolysis effect, other factors might also influence the longevity of fiber posts bonding. Besides, the incorporation of chlorhexidine into ED primer might change the features or performances of the primer (*i.e.* degree of conversion, degree of polymerization, mechanical properties), which might be another reason leading to the bond strength reduction of 1.0% group.

CONCLUSION

The incorporation of 1.0% chlorhexidine into ED primer of Panavia F can extend the bond longevity of fiber post to radicular dentin.

ACKNOWLEDGMENTS

The authors are grateful to Kuraray Co., Ltd. for the generous donation of Panavia F. We are also grateful to Mr Zhihui Sun and Mr Wei Bai (Department of Dental Material, Peking University School and Hospital of Stomatology, Beijing, P. R. of China) for their assistance in the push-out test.

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