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Effect of Carbodiimide on Bonding Durability of Adhesive-cemented Fiber Posts in Root Canals

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Clinical Relevance

Pretreatment of root dentin with carbodiimide cross-linker prior to fiber-post adhesive cementation improves resin-dentin interface durability.

SUMMARY

This study was undertaken to investigate whether using a protein cross-linker, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), improves bonding stability of fiber posts to root dentin using three resin cements. Sixty human maxillary central incisor roots were randomly divided into six groups after endodontic treatment, according to the cements used with and without EDC pretreatment. In the etch-and-rinse group, 0.3 M EDC aqueous solution was applied on acid-etched root dentin prior to Excite DSC/Variolink II for post cementation. In the self-etch and self-adhesive groups, EDC was used on EDTA-conditioned root space prior to application of ED Primer II/Panavia F2.0 and Clearfil SA, respectively. After microslicing the root dentin, a push-out bond strength (BS) test was performed immediately or after one-year of water storage for each group. Data were analyzed using three-way analysis of variance and Tukey tests (α=0.05). A significant effect of cement type, time, EDC, and Time $\times$ Cement and Time $\times$ EDC interactions were observed (*p*$\leq$0.001). EDC pretreatment did not affect immediate bonding of the three cements (*p*$>$0.05). Aging significantly reduced the BS in all the groups (*p*$\leq$0.001), but EDC groups exhibited a higher BS compared with the respective control groups (*p*$<$0.001). Despite the significant effect of aging on decreasing the BS of fiber post to radicular dentin, EDC could diminish this effect for the three tested cements.

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INTRODUCTION

Fiber-reinforced composite posts are commonly recommended to provide retention of the final restoration in endodontically treated teeth due to many favorable properties.[1] In addition to esthetic advantages, elastic modulus similar to dentin and adhesive bonding ability of glass fiber posts to root canal dentin could distribute stress more homogeneously in the root, reducing risk of vertical root fracture.[2,3] Despite promising clinical performance of fiber post–reinforced restorations, loss of post retention has been described as the most common failure of the restorations.[1,4] This could be associated with failure of endodontic treatment.[5]

In adhesive cementation of fiber posts, establishment of a highly durable bond between resin cement and root dentin is an essential factor to provide a coronal seal and adequate retention.[1,5] Not only is initial bonding to root dentin obtained with difficulty,[1,6] but also it is exposed to degradation mechanisms over time.[5] One of the most important mechanisms is the degradation of exposed collagen by matrix metalloproteinases (MMPs) and cysteine cathepsins at the adhesive interface in radicular dentin[7,8] similar to coronal dentin.[9-11] Chlorhexidine as a nonspecific MMP and cathepsin inhibitor could prevent dentin bond degradation.[12-17] Despite reports on the positive effect of chlorhexidine on the durability of radicular dentin bonding, the effect has not been confirmed by some authors.[18,19] Water solubility and electrostatic reversible bond of chlorhexidine to collagen might lead to its slow diffusion from collagen matrix, resulting in loss of MMP-inhibitory effectiveness within 18-24 months.[12,20]

A stable isomer of carbodiimide, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), is a newer nonspecific protein cross-linker with low cytotoxicity.[12,21] It has been shown to be capable of inactivating the catalytic sites of MMPs and cathepsins. This occurs via activating the carboxyl groups and cross-linking of amino acids, creating new, stable covalent peptide bonds and the resultant reduced molecular mobility. The mobility is mandatory for collagenolytic activity of the enzymes.[12,22,23] The cross-linking is very rapid due to greater accessibility of reactive groups of MMPs than of exposed collagen within a one-minute application time.[22] This mechanism of MMP inhibition could last much longer than for the matrix-bound mechanism of chlorhexidine.[24] The preservative effect of EDC on long-term bond strength (BS) of etch-and-rinse (E&R) adhesives to coronal dentin has been recently reported.[25]

The aim of this study was to test the null hypothesis that EDC pretreatment of root dentin has no effect on the bond longevity of fiber post to radicular dentin luted using different types of adhesive resin cement.

METHODS AND MATERIALS

Specimen Preparation

Sixty sound human maxillary central incisors with similar size and anatomic shape as well as straight roots without cracks were selected and stored in 0.5% chloramine-T solution at 4°C until use. They were used following informed consent from patients and approval of the research protocol by the local ethics committee. The roots were separated from the crowns in a uniform length of 15 mm, using a water-cooled diamond saw (D&Z, Berlin, Germany) at the cementoenamel junction. The roots were endodontically instrumented at a working length of 1 mm from the apex with K-files (Dentsply Maillefer, Ballaigues, Switzerland) up to No. 50 with saline solution and 2.5% sodium hypochlorite irrigation. The roots were obturated using AH26 sealer (Dentsply Caulk, Milford, Germany) and gutta-percha (Aria Dent, Asia Chemi Teb, Tehran, Iran) and were coronally sealed using light-cured glass ionomer (Fuji II LC, GC Corporation, Tokyo, Japan).

The specimens were stored in water for one week for complete setting. Afterward, post spaces were prepared to a standardized depth of 10 mm using No. 2 drills from the respective post manufacturer by the same operator. Cleanliness of root walls and the remaining 4 mm of gutta-percha at the root end for apical seal were confirmed by radiographs.

Fiber posts (FRC Postec Plus No. 2, Ivoclar Vivadent, Schaan, Liechtenstein) were tried in the canals for a passive fit to the prepared depth. Post surfaces were cleaned with ethanol, air-dried, and then silanized.

The specimens were divided into six groups according to the resin cements and whether EDC was used (n=10). In three control groups, etch-and-rinse cement (E&R; Excite DSC/Variolink II, Ivoclar Vivadent), self-etch cement (SE; ED Primer II/Panavia F2.0, Kuraray, Osaka, Japan), and self-adhesive cement (SA; Clearfil SA, Kuraray) were used according to manufacturers' instructions (Table 1). For self-etch and self-adhesive cement, the root space was irrigated with 1 mL of 18% EDTA for one minute to remove debris and the remaining obturating materials, rinsed for one minute, and dried. In the three experimental groups, the same cements were used and EDC pretreatment was included in the adhesive cemen-

Table 1: *Composition and Application Mode of the Used Resin Cement*

Resin Cement/ Manufacture (Lot No.)	Resin Cement Type	Application Mode	Composition
Excite DSC + Variolink II, Ivoclar Vivadent, Schaan, Liechtenstein (S08885+P72405)	Etch-and-rinse (E&R)	Phosphoric acid etching for 15 s, rinsing, gently air drying, applying Excite DSC for 10 s, removing the excess, gently drying, cementing post with mixed Variolink II, light curing for 40 s.	Excite DSC: phosphonic acid acrylate, dimethacrylates, HEMA, highly dispersed silicon dioxide, ethanol, catalysts, stabilizers, fluoride microbrush: coated with initiators
			Variolink II: Bis-GMA, UDMA, TEGDMA, barium glass filler, ytterbium trifluoride, Ba-Al-fluorosilicate glass, catalyst, stabilizers, pigments
ED Primer II + Panavia F2.0; Kuraray, Osaka, Japan (A: 00198A, B: 00324F + A: 00588A, B: 00116C)	Self-etch (SE)	Mixing ED primer II, applying for 30 s, gently drying, cementing with mixed Panavia F2.0, light curing for 40 s.	ED primer II: A—HEMA, MDP, chemical initiator, water, 5-NMSA; B—5-NMSA, chemical initiator, water
			Paste A: hydrophobic aromatic and aliphatic dimethacrylate, sodium aromatic sulfinate, *N,N*-diethanol-*p*-toluidine, functionalized sodium fluoride; glass paste B: 10-MDP, hydrophobic aromatic and aliphatic photoinitiator, dibenzoyl peroxide dimethacrylate, hydrophilic dimethacrylate, silanized silica
Clearfil SA; Kuraray, Osaka, Japan (00423A)	Self-adhesive (SA)	Cementing with mixed Clearfil SA and light curing for 40 s.	Bis-GMA, TEGDMA, MDP, silanated filler, hydrophobic aromatic and aliphatic dimethacrylate, benzoyl peroxide, DL-camphorquinone, initiator, silanated fillers, surface-treated, sodium fluoride, accelerators, pigments

Abbreviations: BisGMA, bisphenol A glycidyl methacrylate; HEMA, 2-hydroxyethyl methacrylate; MDP, 10-methacryloyloxydecyl dihydrogen phosphate ; 5-NMSA, N-methacryloyl 5-aminosalicylic acid; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate.

tation of the post. In the E&R group, acid-etched root dentin was pretreated with 0.3 M EDC aqueous solution (Sigma-Aldrich, St Louis, MO, USA) for 60 seconds. The bonding procedure was similar to that of the corresponding control group. The same EDC pretreatment was used in the EDTA-conditioned root dentin prior to the application of ED primer II in the SE group or prior to SA cementation.

In each group, the respective cement was applied on the post surface and to the post space. The post was immediately seated with a slight vibratory motion and held under finger pressure. After removing the excess cement, light polymerization was carried out for 40 seconds at 600 mW/cm^2 using a light-curing unit (VIP Junior, Bisco, Schaumburg, IL, USA) according to the manufacturer's instructions. Finally, a tight coronal seal was obtained using Fuji II LC. The specimens were stored in distilled water at 37°C for one week.

Push-out Test and Failure Mode Analysis

The bonded roots were sectioned into seven 1-mm-thick slices by using a slow-speed cutting machine (Mecatome T201 A, Presi, Grenoble, France. In each root, two slices from each root region (apical, middle, and coronal) were obtained. The first coronal slice was not included.

In half of the roots from each group (n=5 roots, 30 slices), the push-out test was performed immediately. The other half of each group was stored in 37°C distilled water containing 0.4% sodium azide for one year before assessing the long-term BS.

The slices were submitted to a compressive load in a universal testing machine (Zwick, Roell, Ulm, Germany) at 0.5 mm/min on the center of the post in an apico-coronal direction until the shear stresses along the bonded interface dislodged the post.

The load in newtons (N) was divided by the bonded interface area (mm^2). The BS was recorded in MPa through the formula π (R + r) $[h^2 + (R - r)^2]^{0.5}$, where R and r represent the coronal and the apical

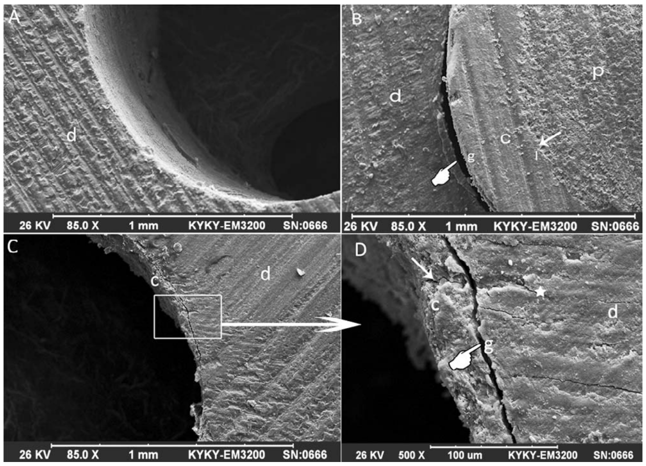

Figure 1. *Scanning electron micrograph of representative failure modes. (A and B): Adhesive failure between the resin cement and dentin, (A): showing no remaining resin on root dentin wall and (B): showing a gap between the resin cement and dentin (pointer) and a gap-free interface between the resin cement and post (l, arrow). (C and D): Mixed failure with the covering cement on the root dentin wall along with separation from the dentin wall (pointer) and cracks in dentin (asterisk) and resin cement (arrow). d, dentin; p, post; c, cement; g, gap.*

post radii, respectively, and h is the thickness of the slice.

Failure Mode Evaluation

All the debonded specimens were assessed under a stereomicroscope (Carl Zeiss Inc, Oberkochen, Germany) at 40× and categorized as follows: 1) cohesive failure in the dentin; 2) cohesive failure in the cement; 3) adhesive failure between the cement and the dentin; 4) adhesive failure between the cement and the post; and 5) mixed failures consisting of combination of two or more failure modes.

The representative specimens of failure modes were prepared for scanning electron microscopy (SEM; EM3200, KYKY, Beijing, China) evaluation of the failure pattern as shown in Figure 1.

Statistical Analysis

Data were statistically analyzed using a three-way analysis of variance (ANOVA) and Tukey multiple comparisons. For each time interval, a one-way ANOVA and Tukey tests were used to compare the BS from different root regions ($\alpha=0.05$).

RESULTS

The mean push-out BS and standard deviations (SD) in MPa are presented in Table 2. The effect of cement type, EDC treatment, time, and interactions between cement and time and between treatment and time were significant ($p \leq 0.001$). Interactions among all three factors (cement, EDC, time) and between cement and EDC treatment were not significant ($p > 0.05$).

SA cement showed a significantly higher immediate BS compared with those of E&R ($p=0.001$) and SE cement ($p=0.02$). EDC treatment did not interfere with the BS of the three cements ($p > 0.05$). Regardless of EDC, all the groups exhibited a significantly lower BS after aging ($p \leq 0.001$). However, after aging, the BS of EDC-treated groups was significantly higher than that of the corresponding control groups ($p < 0.001$). After aging, there were no significant differences among the three EDC groups, but the SE control group exhibited a lower BS compared with that of the E&R control group ($p=0.017$), with no difference from the SA cement ($p=0.59$).

The mean BS and SD in MPa from different root regions are presented in Table 3. In all the groups, progressively decreasing push-out strengths were recorded at the coronal, the middle, and the apical regions, respectively. Among immediate groups, in E&R (control and EDC), in SE (control), and in SA (control and EDC), the differences were not statistically significant ($p > 0.05$). When analyzed regionally, there were no significant differences in

Table 2: *Long-Term Bond Strength [Mean (Standard Deviation), MPa] for Resin Cement and EDC Dentin Treatment at Two Aging Periods (n=30)*

Cement	Treatment	Immediate	1-year Aging	*p*-value*
Variolink II	Control	15.52 (2.7) A[a]	9.11 (1.9) C	<0.001
	EDC	15.11 (2.9) A	12.12 (2.1) A	<0.001
Panavia F2.0	Control	16.24 (2.4) AB	7.40 (2.1) B	0.001
	EDC	16.07 (2.6) AB	13.21 (1.7) A	<0.001
Clearfil SA	Control	18.33 (2.2) C	8.91 (2) BC	<0.001
	EDC	17.92 (2.1) BC	13.50 (2.8) A	<0.001

Abbreviation: EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide.
[a] Different small cap letters in columns indicate a statistically significant difference at significance level of 0.05.
** A significant difference between immediate and 1-year aged value for each group.*

Table 3: *Bond Strength [Mean (Standard Deviation), MPa] for Different Root Regions (n=10)*

Cement	Treatment	Coronal		Middle		Apical	
		Immediate	1-year	Immediate	1-year	Immediate	1-year
Variolink II	Control	16.14 (3.1) A,A[a]	9.90 (1.8) B,A	15.86 (2.83) A,A	9.29 (2.1) C,AB	14.61 (2.6) AB,A	8.26 (2.2) CD,B
	EDC	15.99 (3.1) A,A	13.11 (2.3) A,A	15.27 (3.0) A,A	12.01 (2.3) A,AB	14.19 (3.1) A,A	11.25 (1.8) AB,B
Panavia F2.0	Control	17.50 (2.2) AB,A	8.06 (2.3) B,A	16.49 (2.5) AB,AB	7.59 (2.3) C,A	14.84 (2.8) AB,B	6.68 (2.2) C,A
	EDC	17.37 (2.6) A,A	13.47 (2.2) A,A	16.24 (2.6) A,AB	12.38 (2.1) AB,B	14.61 (2.9) AB,B	10.96 (2.1) A,C
Clearfil SA	Control	20.20 (2.4) C,A	8.96 (2.0) B,A	19.23 (2.0) C,B	8.91 (1.2) C,A	16.17 (2.5) B,C	8.87 (2.1) D,A
	EDC	19.68 (2.3) BC,A	14.30 (3.2) A,A	18.17 (2.4) BC,B	13.84 (2.8) B,A	16.07 (2.5) AB,C	12.62 (2.6) B,A

Abbreviation: EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide.
[a] For each interval (immediate and 1-year), different uppercase letters indicate statistically significant difference within each column (different subgroups), and different small cap letters indicate statistically significant difference within each line (different root regions) ($p<0.05$).

immediate BS between the control and EDC group for the three cements in the three root regions ($p>0.05$). After aging, the results of comparisons between the control and EDC group for three cements in all the root regions were similar to the results analyzed totally.

The results of failure modes of the six study groups are reported in Table 4. The majority of failure modes were mixed failures in immediate groups, followed by adhesive failures between the post and cement. After aging, the bond failures were mainly adhesive between the dentin and cement, except for EDC groups with predominantly mixed failures.

DISCUSSION

According to the results of this study, despite a significant reduction of BS after aging in all the groups, EDC treatment could diminish the loss of BS with all three cements; as a result, aged EDC-treated groups exhibited significantly higher BS compared with the aged nontreated groups (results of total and regional analysis in Tables 2 and 3). Therefore, the tested null hypothesis was rejected. These findings might be attributed to degradation of both the resin and collagen during the accelerated aging used in this study via direct water exposure of microsliced specimens. Sectioning of bonded roots before water storage might lead to rapid water diffusion through the small surface area of the adhesive interface, resulting in a rapid degradation process.[26] This has been used in some studies on bonding longevity of fiber posts,[18,27] whereas the full lengths of bonded roots have been water-aged in others.[16,19] Long-term water storage and subsequent exposure of root microslices to dislodging forces during the push-out test cannot closely mimic clinical aging conditions and functional loads. However, this experimental set-up was previously designed to examine the role of anti-MMP agents in bonding stability of fiber posts to radicular dentin.[27]

Table 4: *Distribution of Failure Mode in the Six Study Groups (n=30)*

Groups	Aging Condition	Failure Mode				
		1 Cohesive in Dentin	2 Cohesive in Cement	3 Adhesive in Cement-Dentin	4 Adhesive in Cement-post	5 Mixed
Variolink II/Control	Immediate	3	1	2	2	22
	1 year	1	—	17	—	12
Variolink II/EDC	Immediate	4	1	2	3	20
	1 year	3	—	10	2	15
Panavia F2.0/Control	Immediate	2	1	3	3	21
	1 year	—	—	20	1	9
Panavia F2.0/EDC	Immediate	5	1	4	1	19
	1 year	2	—	9	3	16
Clearfil SA/Control	Immediate	6	2	—	2	20
	1 year	1	—	14	1	14
Clearfil SA/EDC	Immediate	3	1	2	—	24
	1 year	3	—	8	4	15

Abbreviation: EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide.

The beneficial effect of EDC pretreatment on bonding stability could be attributed to its MMP inhibitory property. However, the role of collagen cross-linking and the strengthening effect of EDC in preventing the loss of bond strength cannot be completely ruled out, especially in the acid-etched dentin in the E&R group. A one- to four-hour treatment time with 0.3 M EDC was previously reported to be required for a collagen stiffening effect.[28] More recently, Scheffel and others[29] demonstrated that 0.5, 1, and 2 M for only 30 seconds were capable of increasing collagen stiffness, preventing unwinding and subsequent cutting of the peptide chains by MMPs.[30] Also, inactivation of dentin proteinases in demineralized dentin matrices in one minute has been recently well documented using zymographic and colorimetric analyses[21-24,29] and indirectly using a bonding durability test.[25]

During E&R or SE bonding procedures, activation of MMPs and cathepsins cause coronal or radicular dentin collagen degradation.[7,11] This activation may occur by the initial low pH of self-adhesive cements or their residual unpolymerized acidic monomers.[31,32]

In the current study, post spaces were irrigated using EDTA before SA and SE cement application. This step might be advantageous with respect to the removal of a thick secondary smear layer and exposure of dentinal tubules, allowing SA cement and SE primer to infiltrate sufficiently into the radicular dentin.[33,34] EDTA pretreatment might be capable of demineralizing dentin adequately to allow cleavage of the exposed collagen.[35] EDTA was found to have MMP inhibitory effects.[32,36] During bonding procedures, large amounts of EDTA were removed from the EDTA-treated dentin by extensive rinsing with water. Therefore, there was no residual EDTA to inhibit the activity of MMPs.[35] Considering the water rinsing after EDTA irrigation used in this study, the adhesive cementation might benefit from the smear-layer-removing effect of EDTA. The MMP inhibitory effect of EDC on EDTA-treated root dentin bonded with SA and SE cement was found in a similar manner with E&R cement after water aging. This inhibitory effect was also supported by failure mode analysis. The number of adhesive failures between the dentin and cement in aged EDC-treated groups was less than that in the aged control groups (Table 4).

It was shown that using a post and resin cement combination from the same manufacturer could minimize possible incompatibility between the materials.[37] In the present study, one post type (Vivadent) was used because the manufacturer of one of the cements did not have a post system.

Among the three adhesive cements used according to different bonding strategies, SA cement exhibited the highest immediate strength. E&R and SE cements require adhesive pretreatment. E&R may exhibit difficulty in the adhesion procedures such as homogeneous etching, complete acid washing, optimal dentin wetness, complete resin penetration, uniform application, and solvent evaporation from the primer/adhesive in narrow and deep root canal spaces with limited access.[19,38,39] The last two issues are also relevant for the SE cement. These limitations may explain the lower immediate bond strength of E&R and SE cement compared with that of SA cement and might impair long-term bonding.[12,40,41] SA cement without pretreatment could solve these problems and be less sensitive to moisture.[41] SA cement contains the phosphoric methacrylated monomer 10-methacryloyloxydecyl dihydrogen phosphate (MDP), with a capacity to form a chemical bond with hydroxyapatite. Low shrinkage stress of SA cements in a confined root space with high C-factor might be a positive property.[41] However, bond strength of the three cements significantly decreased after aging with no significant differences between the SA and the others. In addition to collagen degradation, water sorption and hydrolysis of hydrophilic ionic resin monomers in the simplified primer/adhesive could account for the instability of the adhesive interface. The primer/adhesive acts as a permeable membrane, facilitating water movement and accelerating degradation and leaching of resin components.[12,40,42,43] The relatively high hydrophilic acidic nature of SA cements[31,44] might contribute to water uptake and hydrolytic degradation of the SA-radicular dentin interface during storage. EDC could not affect the resin deterioration process. This pretreatment introduces an additional step to the cementation procedure. EDC might be incorporated into adhesives, reducing chair time. The effectiveness of EDC treatment may be adhesive dependent;[25,28] this effect on more hydrophobic adhesive blend interfaces should be tested.

The results of comparisons made between the push-out BS of different root regions revealed that the coronal region had higher BS than the middle region and the apical region, although the differences were not statistically significant in all the groups. A significantly or insignificantly higher BS in the coronal region[15,19,27,45] or comparable BS for the three regions[44] was found in the literature.

EDTA irrigation was carried out before SE and SA cement application to remove the smear layer, thereby facilitating contact between EDC and the exposed root dentin in EDC-treated groups. Other irrigant solutions with antimicrobial effects (ie sodium hypochlorite and chlorhexidine) have been suggested before post cementation.[45-48] The possible interactions between these irrigants and EDC might result in different effects on the longevity of cemented fiber posts, necessitating further studies.

CONCLUSION

Considering the limitations of the present study, EDC treatment of root dentin was capable of improving longevity of the resin-dentin interface for the three types of resin cements over time.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the School of Dental Medicine, Shiraz University of Medical Sciences in Shiraz, Iran.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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