

Efficiency of Post-Space Irrigation Protocols on the Push-Out Bond Strength of Fiber Post to Post-Space

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Post Boşluğu İrrigasyon Protokollerinin Fiber Post ile Post Boşluğu Arasındaki İtme Bağlanma Dayanımı Üzerindeki Etkinliği

ABSTRACT

Objective: This study aimed to examine how different post-space irrigation protocols influence the push-out bond strength (PBS) of fiber posts to the post-space.

Method: The study involved 60 mandibular first premolar teeth with single root. The teeth were divided into two in accordance with the irrigation protocol (n = 30): passive irrigation and sonic irrigation. A self-adhesive resin cement was used for the cementation of fiber posts. One half of the specimens underwent chewing simulation for 250,000 cycles of dynamic loading with thermal cycling. For each tooth, three transversal sections were taken, with each section having a thickness of 2 mm. These sections were collected from the coronal, middle, and apical thirds of the post-place. The PBS in each section was examined using a universal testing device. The collected data were subjected to statistical analysis using three-way ANOVA and Tukey tests, with a significance level set at 5%.

Results: The passive irrigation group demonstrated the lowest bond strength ($P < .05$). The coronal region exhibited elevated bond strength compared to the apical region, irrespective of the irrigation protocol ($P < .05$). The sonic irrigation group predominantly exhibited mixed failure, whereas the passive irrigation group mainly exhibited adhesive failure type. Additionally, the bond strength values decreased significantly after the chewing simulator.

Conclusion: Regardless of the irrigation protocol, higher fiber post bond strength values were obtained for the coronal third compared to the apical third. Sonic activation resulted in a twofold increase in PBS in the apical third compared to passive irrigation.

Keywords: Adhesion, Bond strength, Endodontically treated teeth, Fiber posts, Irrigation.

Öz

Amaç: Bu çalışmanın amacı, farklı post boşluğu irrigasyon protokollerinin fiber postların post boşluğuna itme bağlanma dayanımını (PBS) nasıl etkilediğini incelemektir.

Yöntem: Çalışmaya tek köklü 60 alt birinci premolar diş dahil edildi. Dişler irrigasyon protokolüne göre ikiye ayrıldı (n = 30): pasif irrigasyon ve sonik irrigasyon. Fiber postların simantasyonu için self-adhesive reçine siman kullanıldı. Örneklerin yarısı, termal döngü ile 250.000 dinamik yükleme döngüsü için çiğneme simülasyonuna tabi tutuldu. Her diş için, her kesitin kalınlığı 2 mm olan üç transversal kesit alındı. Bu kesitler post boşluğunun koronal, orta ve apikal üçte birlik kısımlarından toplandı. Her kesitteki PBS, universal test cihazı kullanılarak incelendi. Toplanan veriler, %5'lik bir önem seviyesi ile üç yönlü ANOVA ve Tukey testleri kullanılarak istatistiksel analize tabi tutuldu.

Bulgular: Pasif irrigasyon grubu en düşük bağ dayanımı gösterdi ($P < .05$). Koronal bölge, irrigasyon protokolünden bağımsız olarak apikal bölgeye kıyasla daha yüksek bağ dayanımı gösterdi ($P < .05$). Sonik irrigasyon grubu ağırlıklı olarak karışık başarısızlık tipi gösterdi, pasif irrigasyon grubu ise ağırlıklı olarak adeziv başarısızlık tipi gösterdi. Ek olarak, bağ dayanımı değerleri çiğneme simülatöründen sonra önemli ölçüde azaldı.

Sonuç: İrrigasyon protokolünden bağımsız olarak, apikal üçte birlik kısımla karşılaştırıldığında koronal üçte birlik kısım için fiber postlara daha yüksek bağlanma dayanımı değerleri elde edildi. Sonik aktivasyon, pasif irrigasyona kıyasla apikal üçte birlik kısımda PBS'de iki kat artışla sonuçlandı.

Anahtar kelimeler: Adezyon, Bağlanma dayanımı, Endodontik tedavili dişler, Fiber postlar, İrrigasyon.



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INTRODUCTION

Factors such as trauma, dental caries, incompatibilities in previous restorations, abrasion, attrition, erosion and developmental disorders negatively affect oral health and general health. Although endodontic treatments ensure that such teeth continue to function in the mouth, large caries, fractures and loss of material from endodontic access cavities make the restoration of these teeth difficult.^{1,2} Such teeth with high coronal tooth tissue loss are more fragile compared to vital teeth. The fragility of the devital pulp is attributed to the loss of material during the preparation of the access cavity for endodontic treatment and/or the absence of moisture in the pulp.^{3,4} The application of endodontic posts and cores is one of the most preferred methods when restoring endodontically treated teeth (ETT) with excessive material loss.⁵

Many techniques and restoratives have been recommended for the restoration of ETT. Metallic prefabricated or cast posts have been used for years. More recently, non-metallic posts compatible with tooth color have been produced as a result of increased aesthetic expectations.⁶ Fiber posts stand out with their aesthetic characteristic of light transmission similar to tooth structure and their elastic modulus close to dentin, which decreases the probability of root fracture.^{6,7} Fiber post systems can achieve micromechanical adhesion to root canal dentin through the use of resin cements, resulting in enhanced restoration retention. This process forms a monoblock structure involving the root-cement-post triad, contributing to improved overall stability.^{8,9} This type of post provides a homogeneous integrity when used with composite resin-based bonding cements, which minimizes the risk of post fracture.¹⁰ To ensure successful bonding when using composite resin-based cements, it is essential to implement procedures that remove or modify the smear layer in the root canal. This is crucial to counteract any negative effects caused by the smear layer on bonding.

Organic components in the smear layer can be removed by dissolving them with sodium hypochlorite (NaOCl) irrigation, while inorganic components can be removed by decalcifying with ethylenediaminetetraacetic acid (EDTA).¹¹ In addition, chlorhexidine, which is a strong antibacterial agent, is currently used for final irrigation. Passive irrigation, a traditional method in which solutions are applied with an irrigation needle, is widely used to clean the root canal system.^{12,13} However, chemical agents that dissolve the smear layer may be difficult to deliver by passive irrigation, especially to the apical part of the narrow post place.¹⁴ Therefore, various irrigation protocols have been introduced to increase the expected effect of irrigation. In the manual-dynamic irrigation method, a push-pull motion is applied to a gutta-percha or canal instrument compatible with the canal diameter in the coronal direction and the irrigation solution is activated by the hydrodynamic motion created.¹⁵ Another method used to activate the irrigation solution is ultrasonic systems. This method involves the transfer of acoustic energy through ultrasonic waves from the canal instrument to the irrigation solution in the root canal, generating fluctuations. As a result, the irrigation solution is activated throughout the entire root canal, including the apical region.

The efficiency of various irrigation solutions and protocols in root canal preparation has been investigated in many previous studies. Indeed, there is limited research exploring the impact of activation techniques used for irrigation after post-space preparation on the bond strength of fiber post systems. More studies are needed to comprehensively understand the effects of different activation methods on the bond strength in this context. Therefore, this study aimed to examine how different post-space irrigation protocols influence the push-out bond strength (PBS) of fiber posts to the post-space. The null

hypothesis of the current in vitro investigation was that there would be no difference in the PBS of fiber posts placed after different post-space irrigation protocols.

METHODS

The methodology of this study was approved by the Clinical Research Ethics Committee (Altınbaş University, Istanbul, Turkey) with decision number 2022/146, date: 29.08.2022. The statistical predictive analysis using GPower software (version 3.1; Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) determined that a minimum sample size of 12 was required in each group to achieve 80% power and a 0.05 error level. Consequently, a total of 15 samples were chosen to be included in each group for the study.

Sixty single-canal lower first premolars with root diameters and lengths similar to each other (root length 15-17 mm) extracted as part of routine dental treatments were kept in 0.1% thymol solution. Teeth with caries, previous root canal treatment, open apex, curved or oval root canals were excluded. In order to achieve standardization in root lengths, the coronal part of the teeth was removed with a diamond fissure bur at a distance of 15 mm from the apex under water cooling.

The root canals were mechanically instrumented using a rotary system (Protaper Universal; Dentsply Maillefer, Ballaigues, Switzerland) with instruments up to F2 size. The irrigation was done with 5% NaOCl (Endosolve - HP; IMICRYL, Konya, Turkey) using an side-vent syringe (total of 10 ml). The smear layer was removed during final irrigation using 15% EDTA (E.D.T.A; IMICRYL, Konya, Turkey) for 3 minutes, followed by irrigation with 10 ml of sterile saline solution. Subsequently, the root canals were dried using paper points, and gutta-percha points (Dentsply Maillefer, Ballaigues, Switzerland) were used to fill the root canals. Afterward, the sealing of the root canals was accomplished with AH Plus resin (Dentsply De Trey, Konstanz, Germany). Temporary filling material was applied for coronal sealing. After the endodontic treatment, the teeth were kept in a humid environment at 37°C for 48 hours.

The post-space was carefully prepared using a specialized drill provided with the fiber post set (Reforpost; Angelus, Londrina, P.R., Brazil) with a length of 11 mm, leaving 4 mm of canal filling at the apical end as apical seal. Then, post-space was irrigated according to two irrigation protocols (n = 30).

Passive irrigation protocol: The irrigation was carried out by using a 27-G side-vent irrigation needle and moving it back and forth in the cervical-apical direction for 1 minute, using a total of 2 ml of 5% NaOCl and dried with paper cones.

Sonic irrigation protocol: A total of 2 ml of 5% NaOCl solution was administered into the post-space for 1 minute while irrigation was performed with a sonic irrigation device (EndoActivator System; Dentsply Sirona, York, PA, USA) operating at a frequency of 10,000 Hz per minute using a polymer tip and dried with paper cones.

Fiber posts (Reforpost; Angelus, Londrina, P.R., Brazil), whose outer surface was treated with silane (Monobond N; Ivoclar Vivadent AG, Schaan, Liechtenstein), were luted with a dual-curing self-adhesive resin cement (PermaCem 2.0; DMG, Hamburg, Germany) into the dried post-space. The cement was carefully delivered into the post-space with special in-canal apparatus attached to the end of the resin cement tube and placed in the canal along the length of the fiber-post opened post-space. The excess cement was discarded from the canal opening and polymerized for 20 seconds with an LED curing device (Valo Grand; Ultradent, Salt Lake City, UT, USA). The specimens were kept in distilled water at 37°C in an oven for a duration of 1 week.¹⁶

Chewing simulation (Aging protocol)

As an aging protocol, half of the specimens were subjected to chewing simulation. The specimens were divided into two groups ($n = 15$). The first group was stored in distilled water at 37°C before the PBS test. The second group underwent cycling loading and thermocycling using a chewing simulation device (MOD-Dental, ESRT, Ankara, Turkey). The simulation involved cycling loading ($\times 250,000$) at a frequency of 1.6 Hz and a force of 50 N. Additionally, a thermal process of 5-55°C was applied every 1 minute during the chewing simulation.¹⁶

Push-out bond strength test

For each tooth, three horizontal sections were taken, with each section having a thickness of 2 mm using a high-speed rotating device (IsoMet High Speed Pro; Buechler, Lake Bluff, IL, USA). These sections were collected from three different regions of the root: coronal, middle, and apical, allowing for a comprehensive analysis of the different parts of the root canal. The sections were positioned in a universal testing device (MOD-Dental, ESRT, Ankara, Turkey), and force was exerted on the post surface until the fiber post was detached from the root surface. The testing device's tip, appropriate for the post diameter, moved at a speed of 1 mm per minute during the test. Special pins with a diameter of 0.8, 0.5, and 0.3 mm were used for coronal, middle and apical slices, respectively. After the PBS test, each specimen was examined at $\times 40$ magnification using a stereomicroscope (SMZ745T; Nikon, Tokyo, Japan) and the failure types was determined: Adhesive (D), cement-to-dentin adhesive failure; Adhesive (F), cement-to-fiber post adhesive failure; Mixed, failure with a combination of the first two failure types. Figure 1 displays the failure modes.

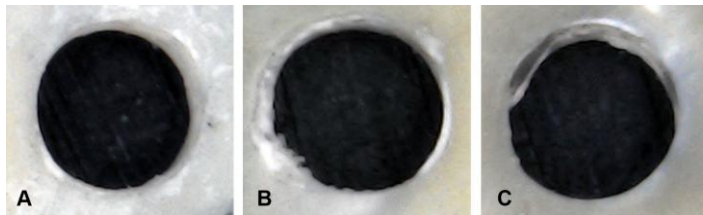


Figure 1. Representative images of failure types. Cement-to-dentin adhesive failure (A), cement-to-fiber post adhesive failure (B), mixed, failure with a combination of the first two failure types (C)

Statistical analysis

The data were analyzed with the Shapiro-Wilk and Kolmogorov-Smirnov tests to assess whether they followed a normal distribution. Three-way ANOVA with Tukey's HSD test was performed for PBS data with factors including irrigation protocol, post-space third, and aging protocol. Additionally, overall PBS values excluding post-space third factor were examined by two-way ANOVA. All tests were conducted using a statistical software (SPSS v29.0; IBM SPSS Corp., Armonk, NY, USA) with a significance level of 5%.

RESULTS

The three-way ANOVA showed that both irrigation protocol ($P < .001$), post-space third ($P < .001$), and aging protocol ($P < .001$) significantly influenced the PBS values (Table 1). Additionally, while significant interactions were observed in the pairwise relationships of these factors ($P < .001$), the three factors together did not cause a significant interaction ($P = .263$). Table 2 displays the mean and standard deviation (in MPa) of the PBS of the fiber post to root dentin in accordance with these three factors. Additionally, Table 3 shows the

overall mean and standard deviation values where the post-space third factor is neglected and the PBS values are grouped according to the other two factors.

Table 1. Influence of irrigation type, post-space third (level), and measurement period (aging) on PBS results according to the three-way ANOVA

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected Model	991.133 ^a	11	90.103	91.390	.000
Intercept	9787.363	1	9787.363	9927.130	.000
Irrigation	377.581	1	377.581	382.973	.000
Level	535.749	2	267.874	271.700	.000
Aging	53.683	1	53.683	54.449	.000
Irrigation * level	8.473	2	4.237	4.297	.015
Irrigation * aging	5.725	1	5.725	5.806	.017
Level * aging	7.267	2	3.633	3.685	.027
Irrigation * level * aging	2.656	2	1.328	1.347	.263
Error	165.635	168	.986		
Total	10944.130	180			
Corrected Total	1156.767	179			

^a R Squared = .857 (Adjusted R Squared = .847)

Table 2. Mean push-out bond strengths (MPa) \pm SD values for experimental groups according to the irrigation protocol, post-space third (level), and measurement period

Irrigation Approach	Level (n)	Immediate (1 week)	After Chewing Simulator	P-Value
Passive irrigation	Coronal (15)	9 \pm 1.5 ^b	7.8 \pm 1.3 ^b	.46*
	Middle (15)	5 \pm 0.7 ^d	5.4 \pm 0.6 ^c	.92
	Apical (15)	3.9 \pm 0.5 ^e	3.5 \pm 1 ^d	.99
Sonic irrigation	Coronal (15)	11.7 \pm 1.2 ^a	9.7 \pm 1.2 ^a	<.001*
	Middle (15)	9.3 \pm 0.9 ^b	8.6 \pm 0.8 ^{ab}	.805
	Apical (15)	7.7 \pm 0.8 ^c	6 \pm 0.5 ^c	<.001*

a,b: Different superscript lower-case letters in the same column imply significant differences within a measurement period (immediate or after chewing simulator) according to the pair-wise analysis of three-way ANOVA ($P < .05$). Asterisk (*) shows statistical difference between measurements with or without chewing simulator ($P < .05$).

Table 3. Mean push-out bond strengths (MPa) \pm SD values for experimental groups according to the irrigation protocol and measurement period (excluding post-space third factor)

Irrigation Approach (n)	Immediate (1 week)	After Chewing Simulator	P-Value
Passive irrigation (45)	6.3 \pm 2.3	5.6 \pm 1.9	.31
Sonic irrigation (45)	9.6 \pm 1.9	8.1 \pm 1.8	.004*
	<.001*	<.001*	

Asterisk (*) shows statistical difference between measurements with or without chewing simulator according to the pair-wise analysis of two-way ANOVA ($P < .05$).

Considering the mean PBS values in the slices obtained from the roots, the coronal region showed a statistically significant elevated mean PBS values compared to the apical region ($P < .05$). The middle region showed similar mean PBS values to the overall values in which post-space third values are combined ($P > .05$). At both measurement times (with or without chewing simulator), the PBS values were significantly elevated in the sonic irrigation protocol than in the passive irrigation protocol ($P < .05$). Additionally, the PBS values decreased significantly after the chewing simulator, except for the middle third, apical third, and overall values in the passive irrigation protocol and the middle third in the sonic irrigation protocol. It is noteworthy that the PBS values in the apical third in the sonic irrigation group were approximately two times higher than in the passive irrigation group.

Failure types of the studied groups are shown in Figure 2. In all study groups, mixed failure was dominant. An elevation in adhesive (D) failure rate and a decrease in mixed failure rates were observed with chewing simulation. The highest rate of mixed failure mode was observed in the

sonic irrigation protocol (immediate 62.2% and after chewing simulator 44.4%). In passive irrigation, failure types are more evenly distributed. The adhesive (D) failure was more prevalent in specimens subjected to passive irrigation especially after chewing simulation (42.2%).

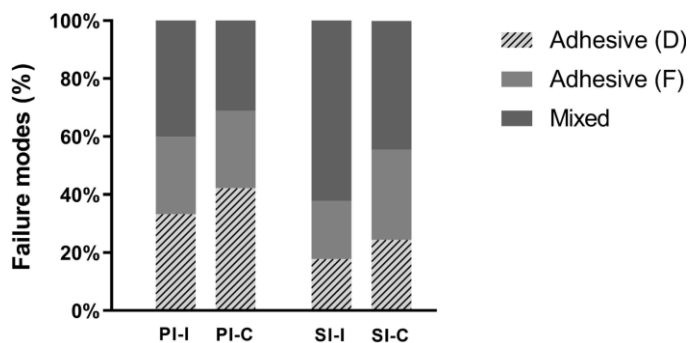


Figure 2. Failure types of the experimental groups

DISCUSSION

Fiber post systems are frequently advised for the prosthetic restoration of ETT with extensive coronal damage due to their mechanical properties, which closely resemble dentin, making them an appropriate option.¹¹ Effective bonding of fiber posts to the root dentin is necessary for achieving successful outcomes over an extended period. Various irrigation protocols have been proposed to improve the bond strength by effectively removing the smear layer on the root dentin after post-space preparation.¹⁷ In the present study, notable variations were observed in the PBSs of fiber posts cemented after employing various irrigation protocols. Additionally, different sections of the post-space showed varying bond strengths, indicating variations in adhesion quality within the post-space. Consequently, the null hypothesis was rejected in light of the study's results.

Resin cements have advantages over conventional cements such as high bond strength and mechanical properties, high resistance to compressive and tensile forces, low solubility, high modulus of elasticity that prevents decementation during function, and aesthetics, but they also have disadvantages such as requiring technical precision during operation and the need for a separate adhesive application step.¹⁸ On the other hand, dual cure resin cements are highly light-dependent.¹⁹ To overcome such problems, manufacturers have recently introduced self-adhesive resin cements that do not require any surface application on the tooth surface and have low technical sensitivity. Research has demonstrated that self-adhesive resin cements display bond strength similar to conventional resin cements.²⁰ Additionally, self-adhesive resin cements were found to offer enhanced bond strength values to root dentin, which was attributed to the better moisture resistance.²¹ According to another study, self-adhesive resin cements were suggested to be more reliable in terms of sealing than conventional resin cements when used without any surface treatment to the tooth.²²

The adhesion quality of restorations bonded directly to dentin can be influenced by anatomical differences in the number and distribution of dentin tubules in different regions of the root dentin.²³ It has been reported that the highest bond strength is achieved in coronal region and the lowest bond strength is apical region, regardless of post type.^{23,24} The reasons for this finding can be summarized as difficulty in reaching the apical part of the post-space, inadequate application of cement to the apical region, residual root canal filling material in the

apical region, decrease in the number of dentin tubules towards the apical region, easier application of bonding agents to the coronal region and the light sources used for polymerization may be more effective in the coronal region compared to the apical region.^{24,25} It is of great importance to evaluate these factors that can lead to failure of post restorations in different regions of the root. In this study, the influence of irrigation protocols on PBS values was analyzed separately for different regions of the root.

In this study, the passive irrigation group exhibited the lowest bond strength value of fiber posts to dentin. In a similar study, improved bond strength values were reported in the group in which the irrigation solution was activated by ultrasonic method in the coronal region compared to passive irrigation, supporting the findings of the study.²⁶ Another study reported that ultrasonic activation of NaOCl positively affected the bond strength between the fiber post and root dentin of some adhesive systems.²⁷ However, there are also studies that reported no difference between ultrasonic and passive irrigation methods in terms of bond strength of fiber posts to root dentin.²⁸ Unlike the present study, EDTA, NaOCl and distilled water were used as irrigation solutions, respectively. The differences between the irrigation solutions in the studies may have an effect on the bond strength. EDTA is an effective inorganic solvent and may be the reason why no difference was observed between irrigation protocols. Previous studies have reported that the combined activation of NaOCl and EDTA caused excessive roughening in the root canals, preventing the formation of hybrid layer with the smear layer, and as a result, negatively affected bond strength.²⁹ Based on the findings, it can be inferred that activating the NaOCl solution without EDTA positively influenced the bond strength of the self-adhesive resin cement between the fiber post and root dentin.

It has been stated that the amount of debris found in the root canal after irrigation with sonic and ultrasonic activation is less than passive irrigation.³⁰ On the contrary, there are also studies that report that ultrasonic activation does not provide any superiority in removing the smear layer and suggest that this result, which contradicts previous research, may be due to differences in canal anatomy.¹⁴ In a study examining the penetration depth of luting resin, it was found that manual-dynamic, sonic and ultrasonically activated irrigation protocols were similar in terms of penetration depth in removing the smear layer in dentin tubules.³¹ In the present study, fiber posts cemented to specimens irrigated by sonic activation method exhibited higher PBS values than passive irrigation.

In this study, the PBS values obtained for the coronal region were dramatically higher than the apical region. There are studies that support this finding.^{11,28} The variations in the PBS of the fiber post in different regions of the root are primarily attributed to variations in the density and distribution of dentin tubules.²⁸ Dentin tubules are denser and wider in diameter in the coronal region, so resin tag formation can be achieved more effectively in the coronal region.^{11,28} In addition, more restricted cement flow to the apical region and incomplete polymerization due to insufficient light transmission may negatively affect the bond strength.¹¹ Although an increase in PBS values was detected, the interaction between the irrigation protocol and the root sections was found to have no significant influence on the PBS of fiber post. This result implies that the coronal region shows superior bond strength regardless of the irrigation protocol applied.

Bond strength evaluations are usually performed 24 hours after restorative procedures and as reported in many studies, long-term clinical use significantly decreases the success rates of restorations.³² In the current study, PBS measurements were performed immediately and

after chewing simulation to evaluate whether bond strength values decrease over time. The immediate measurement results revealed that the bond strength was highest in coronal sections of sonic irrigation and lowest in apical sections of passive irrigation. Similar result was also observed in the measurements made after artificial aging. However, the results obtained after chewing simulation in the sonic activation group were higher than the immediate results of passive irrigation group. A previous study explored the influence of passive, manual-dynamic, sonic, and ultrasonic irrigation protocols on the bonding of fiber posts to root dentin.²⁴ Following post-space preparation, the utilization of manual dynamic, ultrasonic, or sonic irrigation protocols improves the PBS values of fiber posts when compared to passive irrigation. Consistent with the findings of the current study, regardless of the irrigation protocol applied, the PBS of the fiber post was reported superior in the coronal third compared to the apical third.

In the present study, three types of failure were observed during the examination of specimens under a stereomicroscope after the PBS test. These included adhesive failure, where the cement separated from either the dentin (adhesive D) or the fiber post (adhesive F), and mixed failure, where both adhesive failures were present together.²⁷ Cohesive failure, reported by other researchers, was not encountered in our study and thus not included in the failure type classification.³³ In the sonic activation group, a significant proportion of mixed failure was observed, whereas the passive irrigation group showed a higher rate of adhesive failure. The presence of adhesive failure, where the bonding cement separates from the dentin surface, indicates poor bond strength.²⁷ The findings from the failure types align with the results obtained from the PBS.

Microtensile, shear, push-out and pull-out tests have been recommended to measure the bond strength of dental materials to root dentin. The push-out test is commonly used to measure the bond strength of the post, and it is considered more reliable than the pull-out test. The push-out test better mimics clinical conditions and reduces the occurrence of premature failure during sample preparation.³⁴ Furthermore, push-out tests generate parallel force at the adhesive interface and provide a more precise measurement of bond strength values than other methods.³⁵ Hence, push-out test was conducted in the current study to measure bond strength of fiber posts to root dentin.

The use of a single cement type and irrigation solution in our study to standardize the differences between irrigation protocols can be considered among the limitations of the study. Additionally, irrigation devices operating with ultrasonic activation are also used today as irrigation protocol. The fact that this device was not included in the study can be considered among the limitations. Future studies may investigate the effects of different types of resin cements and various combinations of irrigation solutions and activation methods on the bond strength of fiber posts.

CONCLUSION

According to the findings of this laboratory-based study, it is suggested that the use of a sonic system to activate NaOCl after post-space preparation, instead of passive irrigation, can enhance the bond strength of fiber posts to root canal dentin. However, it is important to note that these findings are limited to the laboratory setting and further research is needed to validate these results in clinical settings. Regardless of the irrigation protocol, higher fiber post bond strength values were obtained in the coronal third of the post-space compared to the apical third. Sonic activation resulted in a twofold improvement in bond strength in the apical third compared to passive irrigation.

Ethics Committee Approval: This study was approved by the Clinical Research Ethics Committee of Altınbaş University (Date: 29.08.2022 Decision No: 2022/146).

Informed Consent: Formal consent is not required for this type of study.

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