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Effect of irrigation systems on the bond strength of calcium-silicate-based cement used as pulp barrier in regenerative endodontic treatment

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ABSTRACT

This study aimed to evaluate the effects of irrigation systems on the bond strength of Biodentine, used as a pulp barrier in regenerative endodontic treatment (RET), to the root canal dentin. Following standardized preparation to obtain an immature tooth model, a modified triple antibiotic paste (mTAP) consisting of metronidazole, ciprofloxacin, and clindamycin was applied to the root canals. Teeth were randomly divided into five groups according to the irrigation system ($n = 10$): Group 1, Conventional Syringe Irrigation (CSI); Group 2, Passive Ultrasonic Irrigation (PUI); Group 3, EndoActivator (EA); Group 4, EndoVac (EV); and Group 5, Nd:YAG laser (LSR). After removing the mTAP from the root canals, parallel sections of 1 mm thickness were obtained, with three sections per tooth. Biodentine was applied to the obtained sections, and a push-out test was performed. The data were recorded in MPa, and images of the fracture types were examined. There was no significant difference between the PUI and EV groups ($p > 0.05$), and their respective values, (4.43 ± 0.63) and (4.37 ± 0.47), were greater than those of the other groups ($p < 0.05$). Although the push-out bond strength of the LSR group was higher than those of the EA and CSI groups, there was no difference between EA and CSI groups ($p > 0.05$). The irrigation system type had a significant effect on the push-out bond strength of Biodentine to the root canal dentin in the RET. PUI and EV groups had significantly higher bond strength values than the other groups.

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Introduction

The pulp of immature permanent teeth is at risk for necrosis owing to trauma, dental anomalies, or caries. Although apexification procedures are commonly used in dental practice, they do not allow ongoing root development, similar to that observed in the regenerative endodontic treatment (RET) procedure [1].

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The RET is defined as a biological application that replaces damaged tissues, including the dentin and root structures, in addition to the cells in the pulp–dentin complex [2]. The advantage of this method over other methods is the increase in the root length and thickness of root canal walls [3]. The Ret al. so requires effective disinfection of the root canal. Calcium hydroxide ($Ca(OH)_2$) and antibiotic pastes are frequently used for disinfection. However, antibiotic pastes negatively affect the bond strength of calcium-silicate-based cement used in the RET [4]. The antibiotic paste needs to be removed from the root canal. The bond strength and marginal adaptation of the endodontic material to the dentin to prevent apical or coronal leakage are important factors for the successful implementation of various endodontic treatment procedures [5]. The triple antibiotic paste (TAP) is acidic—if it is not completely removed from the root canal, it causes insufficient bonding of the calcium-silicate-based cement to the root dentin [6].

The intracanal medicaments used in the RET cannot be completely removed from the root canal using the current irrigation systems and solutions [7]. A wide variety of techniques and irrigation solutions have been used to remove antibiotic pastes from mature permanent tooth root canals [8,9]. However, there are limited studies in the literature on the removal of the TAP from the root canal of immature teeth using different irrigation systems.

Calcium-silicate-based cement are used as a pulp barrier in the RET to provide coronal sealing and create resistance toward incoming forces [10–12]. To increase the bond strength of these cement to the dentin, intracanal drugs must be removed from the root canal. Therefore, the investigation of the effects of different root canal irrigation systems used to remove intracanal medicaments using the RET on the bond strength of the calcium-silicate-based cement to the dentin is important for the clinical success of the RET.

The aim of this study was to examine the effects of different irrigation systems by removing intracanal medicaments on the bond strength of Biodentine, a calcium-silicate-based cement used as a pulp barrier in the RET. The null hypothesis of the present study was that the irrigation systems used for removing the modified triple antibiotic paste (mTAP) intracanal medicament used in the RET would have no effect on the bond strength of Biodentine to the root canal dentin.

Materials and methods

Tooth selection

This study was approved by the Health Ethics Committee of Sivas Cumhuriyet University, Turkey (ID:12/08). Fifty single-rooted human maxillary central incisor teeth with periodontal problems were freshly extracted. Multidimensional preoperative radiographs (65 kVp, 8 mA, 0,1 sn, Novelix, Trophy) were taken to confirm the root curvature of less than 20° and the presence of a single, non-complicated root canal. Roots were also examined under an operating microscope (Carl Zeiss AG, Oberkochen, Germany) for visible abnormalities. The teeth were immersed in NaOCI (Wizard, Ankara, Turkey) for 3 h, and the root surfaces were cleaned using a curette. Teeth were stored in 0.1% thymol solution at 4 °C using laboratory procedures.

The sample for each group consisted of 10 teeth, and the power analysis revealed $p = 0.90176$ by using the values based on a previous study [4] and $\alpha = 0.01$, $\beta = 0.10$, $1 - \beta = 0.90$.

Specimen preparation

Each tooth was cut from the enamel junction with a steel separator, and the root length was standardized as 15 ± 1 mm. Following the access cavity, the working length (wl) was determined by inserting a no. 10 K file (Dentsply Maillefer, Ballaigues, Switzerland) into each root canal until apically visible under a 20× magnifying loupe and then subtracting 1 mm from this point. The mechanical preparation of the roots was studied using TF Adaptive (SybronEndo, Glendora, CA, USA) rotary system files, ML1-ML2, and finished apically in ML3 (050.04 tapers).

Root canals were irrigated with 2 mL of 2.5% NaOCl in each rotary file. To provide an immature tooth model with a standard 1.5-mm intracanal diameter, Peeso reamers (Mani Inc., Tochigi, Japan) from #1 to #6 were sequentially used. Finally, the #6 Peeso reamer protruded 1 mm beyond the apical foramen [13]. The prepared teeth were irrigated with 5 mL of 2.5% NaOCl and then with 5 mL of 17% EDTA (Canal + Septodont, Paris, France). Final irrigation was performed with 10 mL of distilled water to remove the effects of the irrigation solutions used in the canal. The root canals were dried with sterile paper cones (Aceonedent, Geonggi-Do, Korea) at the end of the procedure.

Preparation and placement of intracanal medicaments

The mTAP (Metronidazole + Ciprofloxacin + Clindamycin) was prepared using a previously described procedure [14]. The antibiotic paste was mixed with equal volumes of an antibiotic powder, and propylene glycol with polyethylene glycol was used as the pharmacological carrier. The powder-to-liquid ratio of the pastes corresponded to 3:1.

The intracanal mTAP consisted of equal volumes of metronidazole, ciprofloxacin, and cylindamycin (Pfizer, Istanbul, Turkey). The mTAP paste was applied to each root canal using a #40 lentulo. The coronal opening of the teeth was sealed with a small cotton pellet and closed with a temporary filling material (Cavit; 3 M ESPE, Seefeld, Germany). All samples were placed in 100% humidity at 37 °C for 3 weeks.

Irrigation procedure

The specimens were randomly divided into five groups according to the irrigation procedure tested ($n = 10$): Group 1, conventional syringe irrigation (CSI); Group 2, passive ultrasonic irrigation (PUI); Group 3, EndoActivator (EA); Group 4, EndoVac (EV); and Group 5, Nd:YAG laser (LSR).

A standardized irrigation procedure was applied to each tooth. Each tooth had the same total final irrigation time of 2 min with an average rate of 5 mL/min, and the total irrigant volume delivered was 10 mL for each canal. The final irrigation procedure was as follows:

Group 1: CSI

The injector cannula was positioned 1 mm coronal to the working length and irrigated with 5 mL of 2.5% NaOCl for 1 min, and then 5 mL of 17% EDTA (Nazar Kimya Ltd., Istanbul) was applied to the root canal for 1 min. Finally, the canals were irrigated with 10 mL of distilled water.

Grup 2: PUI

The irrigation tip of the VDW ULTRA ultrasonic device (VDW, Munich, Germany) was positioned 1 mm shorter than the working length with an application power of 25 (irrigation mode), in accordance with the manufacturer's instructions. Irrigation was performed in recurrent three rounds of 20 s each. After the root canal was filled with 2.5% NaOCl, the tip of the ultrasonic device was activated for 10 s, a 2 mm up-down movement was performed, and the irrigation solution was renewed for 10 s. Thus, three rounds of irrigation were performed. Irrigation was performed in a manner similar to that conducted for 1 min with 5 mL of 17% EDTA. Finally, the canals were irrigated with 10 mL of distilled water.

Group 3: EA

The power of the EndoActivator (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA) sonic irrigation device was adjusted to 10000 cpm by attaching irrigation tip number 25 and positioning it 2 mm behind the working length. Irrigation was performed in three rounds of 20 s each. After the root canal was filled with 2.5% NaOCl, activation was performed with 3 mm slight back and forth movements for 10 s, and the irrigation solution was renewed for 10 s and waited. Thus, three rounds of irrigation were performed. Irrigation was performed in a manner similar to that conducted for 1 min with 5 mL of 17% EDTA. Finally, the canals were irrigated with 10 mL of distilled water.

Group 4: EV

Irrigation was performed according to the manufacturer's instructions. After active irrigation with 2.5% NaOCI for 30 s with the macrocannula, the root canals were filled with NaOCI, and a total of 30 s of irrigation was performed with the microcannula in 6 s working size, 2 mm shorter than 6 s working size, and 6 s working size. After 30 s of active irrigation with 17% EDTA, the root canals were filled with EDTA and a total of 30 s of EDTA irrigation was performed with a microcannula in 6 s working size, 2 mm shorter than 6 s working size, and 6 s working size, respectively. Finally, the canals were irrigated with 10 mL of distilled water.

Group 5: LSR

A Nd:YAG (Fidelis, Fotona, Ljubljana-Slovenia) laser with a wavelength of 1064 nm adjusted to 1.5 W energy, 100 mJ pulse rate, and 15 Hz frequency, which are the parameters recommended by the manufacturer for endodontic treatment, was placed in the 200 µm fiber optic end channel attached to the laser. Irrigation was performed in three rounds of 20 s each. After the root canal was filled with 2.5% NaOCl, activation was performed with an optical fiber tip for 10 s, and the irrigation solution was renewed for 10 s. During irrigation activation, the optic tip was moved from the apical foramen to the canal mouth with uninterrupted circular movements. Irrigation was performed in a similar manner for 1 min with 5 mL of 17% EDTA. Finally, the canals were irrigated with 10 mL of distilled water.

Placement of cement

L-shaped metal molds with a width of 2 cm and height of 2 cm were prepared before embedding in acrylic molds to obtain sections from the teeth under water cooling with a cutting knife. Silicone impression molds were prepared by measuring the molds with a silicone impression material (Bonasil Putty, DMP LTD, Greece). Cold acrylic was poured into the prepared silicone impression molds, and the teeth were embedded in acrylic so that the apical 2/3 of their roots remained.

Using the IsoMet cutting device (IsoMet 5000, Buehler, IL, USA) at a low speed, three horizontal sections of approximately 1 mm thickness were taken from each tooth from coronal to apical with 0.3-mm-thick diamond discs under water cooling (50 teeth $\times$ 3 sections $=$ 150 sections). Thus, 30 sections were obtained for each experimental group. The thickness of the sections was measured using a digital caliper (Teknikel, Istanbul, Turkey) with an accuracy of 0.001 mm.

Biodentine (Septodont, Saint Maur des Fossés, France) liquid was dropped into the powder-containing capsule (5 drops in total), and the capsule was closed and mixed for 30 s by placing it in an amalgamator (ADM 9002, Medident GbR, Treffurt, Germany). The cement was taken with a plastic spatula and placed in the canal spaces of the horizontal sections, separated into groups on a clean glass surface, and compacted with the help of a hand carrier. Excess material was removed from the surfaces of the samples using the plastic spatula.

The push-out bond strength test was performed after the samples obtained were wrapped in wet gauze in Petri dishes and kept for 1 week at 37 °C in 100% humidity.

Push-out bond strength test

The bond strength test of the specimens was performed using a universal tester INSTRON (Lloyd LRX; Lloyd Instruments Ltd., Fareham, England). A stainless-steel cylindrical tip with a diameter of 0.76 mm was positioned in accordance with the diameter of the spaces in the center of the discs in the experimental groups, only to contact the cement. An acrylic mold was prepared to avoid resistance during the removal of the tested material from the canal when force was applied to the canal filling. A pushing force was applied from the coronal to an apical direction at 1 mm/min until ligament rupture occurred. The force was recorded in newton (N) using the Nexygen data-analysis software (Lloyd, LRX). The following formula was used to convert the values obtained in newton for bond strength to MPa:

$$\text{Bond strength (MPa)} = \frac{\text{Force (N)}}{\text{area} = 2\text{r} \times \text{h}\,(\text{mm}^2)}$$

(π = constant value = 3.14, r = radius of intraradicular space, h = slice thickness in mm).

Analysis of failure modes

Following the fracture process, the fracture surfaces of all the samples were examined using a stereomicroscope (Zeiss, Oberkochen, Germany) at 25× magnification. After stereomicroscope examination, the fracture types of the samples were determined and recorded. Images of the refraction types were acquired using a camera attached (Canon750D, Japan). Fracture types were determined according to the following classifications: (i) cohesive type: fracture is completely within the cement; (ii) adhesive type: fracture is in the adhesive layer between the cement and dentin; and (iii) mixed type: adhesive and cohesive fractures are seen together.

Statistical analysis

Data were analyzed using SPSS (version 22.0; SPSS Inc., Chicago, IL, USA). Kappa was used for intra- and inter-observer agreements. While determining the statistical significance, whether the parametric test assumptions were fulfilled was tested using the Kolmogorov-Smirnov test. Because the bond strength data showed a normal distribution, one-way analysis of variance and Tukey's test were used for pairwise comparisons. Descriptive statistics, including mean ± standard deviation, were also used. The statistical significance level was set at $p < 0.05$.

Results

Table 1 lists the bond strength values (MPa) and fracture types of the experimental groups. The type of irrigation system had a significant effect on the push-out bond values of Biodentine to the root canal dentin ($p < 0.05$). Figure 1 shows the distribution of the fracture types among all the groups. Stereomicroscopic examination

Table 1. Push-out bond strength values (mpa), and distribution of failure modes for each groups.

Irrigation System	Bond Strength (MPa) Mean ± SD	Mode of Failure % (A / C / M)
Passive Ultrasonic Irrigation [a]	4.43 ± 0.63	60.0 / 26.7 / 13.3
EndoActivator[c]	2.83 ± 0.45	63.3 / 30.0 / 6.70
EndoVac[a]	4.37 ± 0.47	53.3 / 30.0 / 16.7
Nd:YAG Laser[b]	3.82 ± 0.47	60.0 / 23.3 / 16.7
Conventional Syringe Irrigation [c]	2.79 ± 0.31	53.4 / 23.3 / 23.3

Same lower case letter represents no statistical significant difference within each column, verified by one-way Anova and Tukey's test ($p > 0.05$). A: adhesive; C: cohesive; M: mixed.

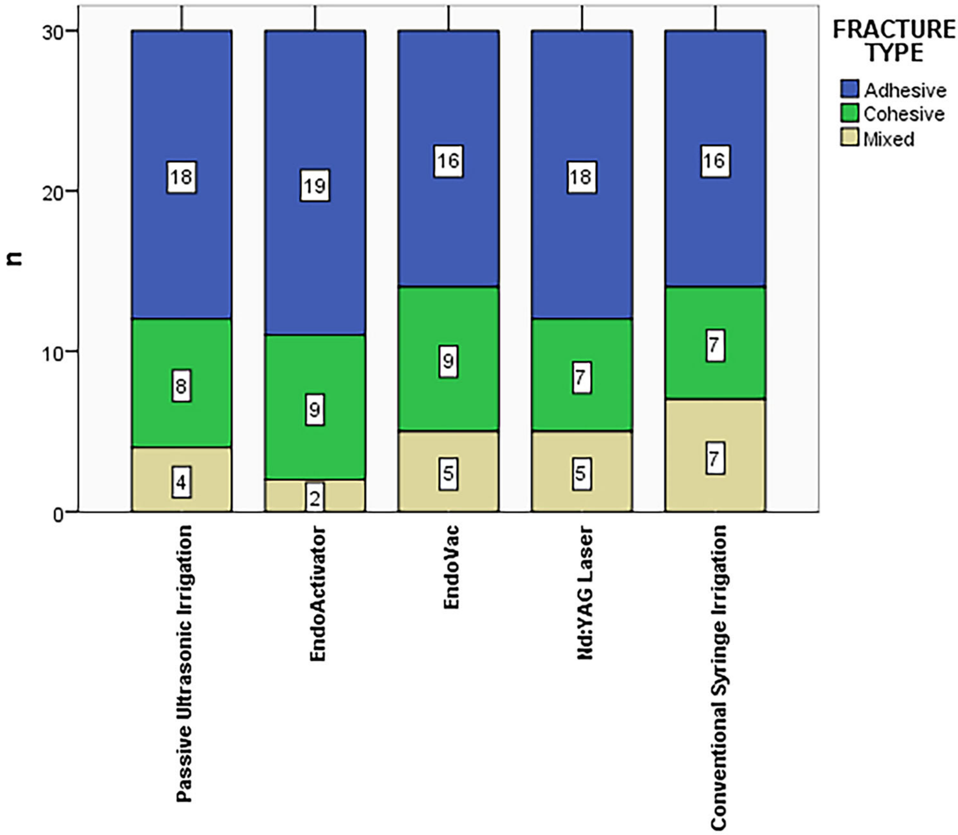

Figure 1. Distribution of failure modes for each group.

revealed that the adhesive-type fracture was the most common fracture type in all groups, while the mixed-type fracture was the least observed fracture type, excluding the CSI group (Table 1 and Figure 1). Figure 2 presents representative stereomicroscope images of failure modes.

Although there was no statistically significant difference between the push-out bond strength values of the PUI and EV groups ($p > 0.05$), the values of both groups were found to be significantly higher than those of the other groups ($p < 0.05$). The push-out bond strength value of the LSR group was found to be statistically significantly higher than those of the EA and CSI groups ($p < 0.05$). Although there was no statistically significant difference between the push-out bond strength values of the EA and CSI groups ($p > 0.05$), the values of the two groups were significantly lower than those of the other groups ($p < 0.05$).

Discussion

The cement used as a pulp cavity barrier in the RET should be resistant to occlusal loads and displacement forces such as the condensation pressure of restorative materials [15]. Therefore, calcium silicate-based cements used as a pulp cavity barrier must show high adhesion to the root canal dentin [16]. As intracanal drugs used in

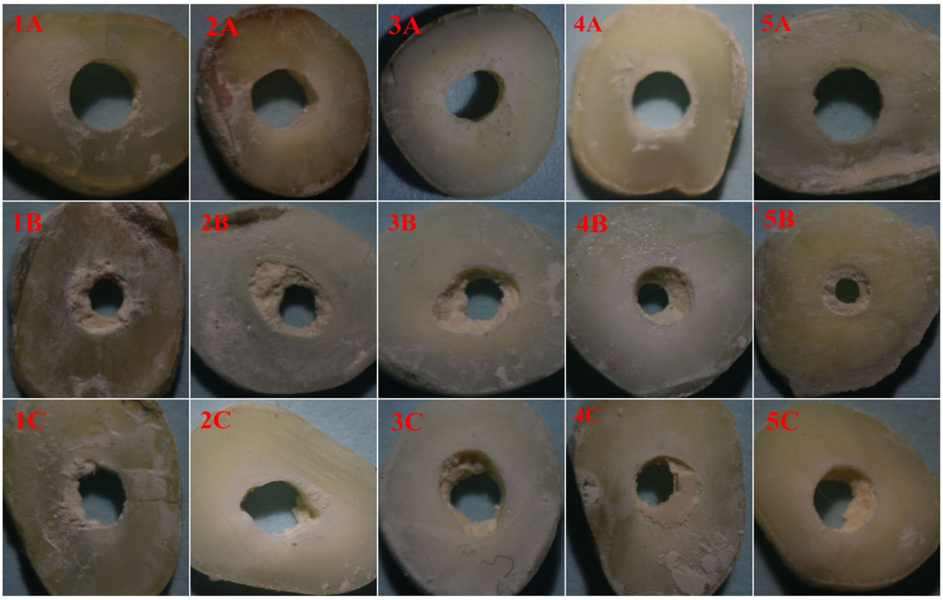

Figure 2. Representative stereomicroscope images of failure modes at ×25 magnification: (1) Passive ultrasonic irrigation; (2) EndoActivator; (3) EndoVac; (4) Nd:YAG Laser; (5) Conventional Syringe Irrigation; A) adhesive failure at cement/dentin interface; (B) cohesive failure within cement, and (C) mixed failure in both cement and dentin.

the RET reduce the bond strength between materials and the root canal dentin and cause tooth discoloration and damage to the stem cells in the apical papilla, they should be removed from the root canal. Several studies have examined the effects of various variables, such as cement type [17,18], intracanal medicaments [4], placement techniques of cement [19], and irrigation regimens [20], on the bond strength of CSCs. However, limited research attention has been paid to the effect of irrigation systems on the bond strength of the CSC to the root canal dentin in the RET. Based on this information and rationale, the effects of CSI, EndoVac, EndoActivator, Nd:YAG Laser, and PUI on the bond strength of the CSC to the root canal dentin were examined in this study. The null hypothesis was rejected because the type of the irrigation system had a significant effect on the push-out bond values of Biodentine to the root canal dentin.

Although several methods [10,21,22] can be used to test bond strength, such as measurement of shear, microshear, tension, and microtension, the push-out test is more advantageous [23]. As the placement conditions of the test materials on the teeth were similar to the clinical conditions in the push-out test, the test conditions and clinical conditions were compared. Because the force applied during the procedure is perpendicular to the dentinal tubules, it clinically mimics the stresses on the tooth. In addition, this test produces less tensile forces at the bonding interface than conventional tensile and shear tests, allowing specimens to be accurately standardized, allowing specimens to be subjected to less shear forces. Based on these rationales, a push-out test was used in this study. Similar to Paulo et al. [18], a cylindrical tip with a diameter og 0.76 mm was used to simulate a 1.5 mm canal diameter.

In accordance with previous studies and methodology [4,10], this study was conducted with extracted human teeth. For the standardization of the imitated immature tooth samples, the root tips of the teeth to be used in the studies were required to be closed, single-rooted, and single-canal. Based on previous studies, the immature tooth model was obtained by working a 1 mm protrusion from the apex with the No. 6 Peeso reamer, and the root lengths of all teeth were fixed at 15 ± 1 mm [13]. All roots were prepared using the TF Adaptive rotary instrument system in accordance with the procedures, and the standardization of apical foramen diameters was achieved.

In this study, a standardized irrigation protocol was applied in accordance with previous studies [13,24]. Root canals were irrigated with 2.5% NaOCI at a flow rate of 5 mL/min for 1 min and then with 17% EDTA at a flow rate of 5 mL/min for 1 min. During the change in the irrigation solutions, while the root canals were filled with the solution, the solution was activated with irrigation activation devices for 10 s. Activation was performed for 30 s. The effects of irrigation solutions associated with distilled water between passes were removed. The root canals were irrigated with 10 mL of distilled water to prevent the prolonged effects of EDTA and NaOCl. Thus, we aimed to obtain fair and reliable findings using a standardized irrigation protocol that followed previous studies and clinical recommendations.

The highest push-out bond strength was recorded for the PUI group. This may be because the PUI technique is based on the transfer of acoustic energy to the irrigation solution [25]. The solution activated by the transferred energy penetrates irregular areas of the canal and facilitates the removal of medicaments. The high flow rate of the solution provided by PUI may also explain its effectiveness for TAP removal [26]. In addition, the continuous flow of fresh solutions increases the efficiency of PUI [27]. These results may explain the high push-out values in the PUI group. Groups that received irrigation activation systems (PUI, EV, EA, and LSR) showed increased bond strength of Biodentine to the root canal dentin in relation to the CSI group. This finding is consistent with those of previous studies [28,29]. Jiang et al. [28] also compared different irrigation systems to remove dentin chips from grooves made artificially in root canals, similar to the current study, and reported that the PUI group showed the highest removal values and the CSI group the lowest.

Stereomicroscopic evaluation showed that adhesive-type fracture was the most observed fracture type in all groups, while mixed-type fracture was the least observed fracture type, except for the CSI group. In a previous study examining the effects of different intracanal medicaments on the bond strength of three different calcium-silicate-based cement to the dentin [4], the most common type of fracture was reported to be the adhesive fracture. The results of this study were consistent with those of the current study. The predominance of the adhesive fracture may be due to the low adhesion of the cement to the root canal dentin [30].

Bond strength values were found to be lower than 5 MPa in all groups. Although these values are lower than the 5 MPa value suggested in the literature, it may be due to the fact that the study was conducted under in-vitro conditions, so it could not fully reflect the *in vivo* environment. Another limitation of this study is that it was designed as an *in vitro* study. Thus, the complete reflection of the oral environment (occlusal stresses, blood-saliva contamination, etc.) was not possible. Therefore, further *in vivo* studies are required to investigate the bond strengths of the tested materials.

Conclusions

In conclusion, the type of irrigation system was confirmed to have a significant effect on the push-out bond values of Biodentine to the root canal dentin in the RET. The PUI and EV groups had significantly higher bond strengths than the other groups. The push-out bond strength of the LSR group was also significantly higher than that of the EA and CSI groups. By using irrigation systems for effective removal, Biodentine, which is used as a pulp barrier in the RET, can increase the bonding strength of the root canal dentin and protect the cement against displacement forces. Therefore, the selection of an appropriate irrigation system should be considered to increase the success of clinical practice.

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Disclosure statement

The authors declare no conflicts of interest.

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Data availability statement

The data used to support the findings of this study can be made available upon request to the corresponding author.

References

[1] Murray PE. Review of guidance for the selection of regenerative endodontics, apexogenesis, apexification, pulpotomy, and other endodontic treatments for immature permanent teeth. Int Endodontic J. 2023;56(S2):188–199. Epub 05 Aug 10.1111/iej.13809.
[2] Hargreaves KM, Diogenes A, Teixeira FB. Treatment options: biological basis of regenerative endodontic procedures. Pediatr Dent. 2013;35(2):129–140.
[3] Nosrat A, Homayounfar N, Oloomi K. Drawbacks and unfavorable outcomes of regenerative endodontic treatments of necrotic immature teeth: a literature review and report of a case. J Endod. 2012;38(10):1428–1434.
[4] Aydin MN, Buldur B. The effect of intracanal placement of various medicaments on the bond strength of three calcium silicate-based cements to root canal dentin. J Adhes Sci Technol. 2018;32(5):542–552.

[5] Diogenes A, Henry MA, Teixeira FB, et al. An update on clinical regenerative endodontics. Endod Topics. 2013;28(1):2–23.
[6] Ribeiro JS, Münchow EA, Bordini EAF, et al. Antimicrobial therapeutics in regenerative endodontics: a scoping review. J Endod. 2020;46(9S):S115–S127.
[7] Berkhoff JA, Chen PB, Teixeira FB, et al. Evaluation of triple antibiotic paste removal by different irrigation procedures. J Endod. 2014;40(8):1172–1177.
[8] Akman M, Akbulut MB, Aydınbelge HA, et al. Comparison of different irrigation activation regimens and conventional irrigation techniques for the removal of modified triple antibiotic paste from root canals. J Endod. 2015;41(5):720–724.
[9] Garcia-Godoy F, Murray PE. Recommendations for using regenerative endodontic procedures in permanent immature traumatized teeth. Dent Traumatol. 2012;28(1):33–41.
[10] Nagas E, Cehreli Z, Uyanik M, et al. Effect of several intracanal medicaments on the push-out bond strength of ProRoot MTA and biodentine. Int Endod J. 2016;49(2):184–188.
[11] Santos JM, Diogo P, Dias S, et al. Long-term outcome of nonvital immature permanent teeth treated with apexification and corono-radicular adhesive restoration: a case series. J Endod. 2022;48(9):1191–1199.
[12] Palma PJ, Ramos JC, Martins JB, et al. Histologic evaluation of regenerative endodontic procedures with the use of chitosan scaffolds in immature dog teeth with apical periodontitis. J Endod. 2017;43(8):1279–1287.
[13] Topçuoğlu HS, Arslan H, Akçay M, et al. The effect of medicaments used in endodontic regeneration technique on the dislocation resistance of mineral trioxide aggregate to root canal dentin. J Endod. 2014;40(12):2041–2044.
[14] Yassen GH, Chu T-MG, Eckert G, et al. Effect of medicaments used in endodontic regeneration technique on the chemical structure of human immature radicular dentin: an in vitro study. J Endod. 2013;39(2):269–273.
[15] Akman M, Akbulut MB, Güneşer MB, et al. Effect of intracanal medicaments on the push-out bond strength of biodentine in comparison with bioaggregate apical plugs. J Adhes Sci Technol. 2016;30(5):459–467.
[16] Kim S, Malek M, Sigurdsson A, et al. Regenerative endodontics: a comprehensive review. Int Endod J. 2018;51(12):1367–1388.
[17] Shokouhinejad N, Khoshkhounejad M, Alikhasi M, et al. Prevention of coronal discoloration induced by regenerative endodontic treatment in an ex vivo model. Clin Oral Investig. 2018;22(4):1725–1731.
[18] Paulo CR, Marques JA, Sequeira DB, et al. Influence of blood contamination on push-out bond strength of three calcium silicate-based materials to root dentin. Appl Sci-Basel. 2021;15:11.
[19] Rodrigues MNM, Bruno KF, de Alencar AHG, et al. Comparative analysis of bond strength to root dentin and compression of bioceramic cements used in regenerative endodontic procedures. Restor Dent Endod. 2021;46(4):e59.
[20] Çelik D, Er K, Serper A, et al. Push-out bond strength of three calcium silicate cements to root canal dentine after two different irrigation regimes. Clin Oral Investig. 2014;18(4):1141–1146.
[21] El-Ma'aita AM, Qualtrough AJE, Watts DC. The effect of smear layer on the push-out bond strength of root canal calcium silicate cements. Dent Mater. 2013;29(7):797–803.
[22] Guneser MB, Akbulut MB, Eldeniz AU. Effect of various endodontic irrigants on the push-out bond strength of biodentine and conventional root perforation repair materials. J Endod. 2013;39(3):380–384.
[23] Collares F, Portella F, Rodrigues S, et al. The influence of methodological variables on the push-out resistance to dislodgement of root filling materials: a meta-regression analysis. Int Endod J. 2016;49(9):836–849.
[24] Dumani A, Yilmaz S, Yoldas O, et al. Effect of irrigation technique for removal of triple antibiotic paste on bond strength of MTA to root dentin. Braz Oral Res. 2016;30(1):e62.

[25] Van der Sluis L, Versluis M, Wu M, et al. Passive ultrasonic irrigation of the root canal: a review of the literature. Int Endod J. 2007;40(6):415–426.
[26] Martin DE, De Almeida JFA, Henry MA, et al. Concentration-dependent effect of sodium hypochlorite on stem cells of apical papilla survival and differentiation. J Endod. 2014;40(1):51–55.
[27] Hariharan V, Nandlal B, Srilatha K. Efficacy of various root canal irrigants on removal of smear layer in the primary root canals after hand instrumentation: a scanning electron microscopy study. J Indian Soc Pedod Prev Dent. 2010;28(4):271–277.
[28] Jiang L-M, Verhaagen B, Versluis M, et al. Influence of the oscillation direction of an ultrasonic file on the cleaning efficacy of passive ultrasonic irrigation. J Endod. 2010; 36(8):1372–1376.
[29] Jiang L-M, Lak B, Eijsvogels LM, et al. Comparison of the cleaning efficacy of different final irrigation techniques. J Endod. 2012;38(6):838–841.
[30] Bouillaguet S, Troesch S, Wataha JC, et al. Microtensile bond strength between adhesive cements and root canal dentin. Dent Mater. 2003;19(3):199–205.