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Evaluating the impact of calcium hydroxide on the push-out bond strength of resin-based endodontic sealers: an in vitro study

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ABSTRACT

Conventional endodontic treatment aims to preserve teeth that would otherwise require extraction through cleaning, shaping, and obturation of the root canal system. Resin-based sealers are multicomponent dental compounds/composites composed of organic resin matrices and inorganic fillers. This in vitro study compared the effect of calcium hydroxide (Ca (OH)₂) on the push-out bond strength of AH Plus and Adseal sealers. A total of 128 single-rooted human teeth were allocated to control (without Ca (OH)₂) and experimental (with Ca (OH)₂) groups. After one week, Ca (OH)₂ was removed using EDTA and NaOCl irrigation with ultrasonic activation, and canals were obturated with AH Plus or Adseal. Following a further week of incubation, specimens were sectioned and subjected to push-out testing. Tooth-level mean values were used for statistical analysis. AH Plus showed significantly lower bond strength after Ca (OH)₂ medication compared with the control group ($p < 0.001$), whereas Adseal was not significantly affected ($p = 0.36$). In the control group, AH Plus exhibited higher bond strength than Adseal ($p = 0.01$); however, in the experimental group, Adseal demonstrated significantly higher bond strength than AH Plus ($p < 0.001$). Within the limitations of this study, Ca (OH)₂ adversely affected AH Plus but not Adseal.

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1. Introduction

Endodontic treatment is a maintenance method for a tooth that will be extracted if left untreated and includes preparing the access cavity, cleaning and shaping, and filling a root canal with gutta-percha [1]. For a successful treatment, a correct diagnosis and a treatment plan are needed, along with debridement, disinfection, and filling of the entire root canal system, which seal the root canal space [1]. The main purpose of root canal filling in endodontic treatment is to completely block the root canal system and create a complete sealing in the apical foramen area, thus providing a suitable biological environment for repairing periapical tissue [2].

The most common method used today for filling the root canal is to use a solid or semi-solid central core cemented by a sealer inside the canal [3]. Among the root canal filling materials, gutta-percha is the most common material used for this purpose. Various studies have shown that root canal filling with gutta-percha and sealer has less microleakage than non-sealer filling [4]. There is a wide range of commercial endodontic sealers on the market that are divided into several groups based on their chemical

composition: sealers based on zinc oxide, epoxy resin, calcium hydroxide, glass ionomer, and calcium silicate [4]. Today, resin sealers have received a lot of attention due to their suitable physical and biological properties, such as their ability to attach to tooth structures, long working time, ease of mixing and preparation of the sealer, and good seal strength. Epoxy resin-based sealers have an excellent apical seal [5]. Endodontic sealers must be able to adhere to dentin structure to prevent bacterial leakage [5]. Improper endodontic sealing can have devastating effects such as prolonged or persistent inflammation and infection [6].

One of the factors that can affect this adhesion is the rinsing solutions and intra-canal drugs used during endodontic treatment, which can intervene with proper filling of the endodontic system [7, 8]. Obstruction of the dentinal tubules by the smear layer or the intra-canal drugs can interfere with the adhesion of endodontic sealers [7]. For this reason, the intra-canal drugs must be removed from the root canal system before filling the root canal so that the best possible intervention occurs between the root canal wall and the filling material [9]. Using the drug inside the canal can improve the root canal condition and disinfect it [10].

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Calcium hydroxide is the most common substance used as an intra-canal drug in the treatment of root canal infection and many types of research have been done on it [11]. Calcium hydroxide is widely used due to its antibacterial and biological properties [12]. Various studies have shown that different methods are not able to completely remove this substance from the root canal system and its removal from the canal is not easy [13-15]. On the other hand, several studies indicate that the residual calcium hydroxide that may remain at the entrance of dentinal tubules and walls can reduce the sealer penetration into dentinal tubules and have negative effects on the bond strength of resin sealers to the dentin wall [16]. AH plus Sealer is an epoxy-based resin sealer that has been used as a standard in several studies to evaluate the seal bond strength [17-19]. According to studies, AH plus sealer is a sealing material with excellent tissue compatibility, and has dimensional stability in a long time, and also has good sealing power [20]. AH plus sealer contains epoxy oligomer resin, ethylene glycol silicate, and bismuth subcarbonate. Adseal is also a resin-based sealer that has recently been introduced to the dental market. According to the manufacturer, it has good tissue compatibility, is insoluble in tissue fluids, and has the power to create a proper seal in the root canal. This sealer has two syringes that contain 9 grams of base (epoxy resin and calcium phosphate) and 4.5 grams of catalyst (amino compound, bismuth subcarbonate) [21]. In light of Adseal's recent introduction to the dental market and since there was no study to investigate the effect of calcium hydroxide residues in the root canal on the strength of Adseal sealer bond to dentin walls, this study was done to fill this gap in knowledge, and this study also aimed to compare the effect of calcium hydroxide on Adseal and AH plus sealers.

2. Materials and methods

2.1. Study design and ethics

This in vitro study evaluated the effect of calcium hydroxide dressing on the push-out bond strength of two resin-based endodontic sealers, AH Plus and Adseal. This study was approved by Bushehr University of Medical Sciences with grant number 522 and by the Ethics Committee of Bushehr University of Medical Sciences (IR.BPUMS.REC.1396.123). This study was conducted in Bushehr, Iran.

2.2. Sample selection and preparation

The study included 128 single-rooted human teeth that were extracted for various reasons. Teeth were selected based on specific inclusion and exclusion criteria. The inclusion criteria required the teeth to be single-rooted, with no root caries or visible canal obstructions, as confirmed by periapical radiographs. Teeth with calcified, obstructed, or multi-canal systems were excluded from the study. Based on Amin et al. [10], the sample size was calculated using a significance level of 0.05 and a statistical power of 80%. The minimum required sample size was determined to be 64 teeth per main group. After extraction and collection from different sources, the teeth were stored in normal saline in sealed containers until use to preserve dentin hydration and minimize dehydration-related changes in dentin properties. Because the teeth were collected at different times from different sources, the pre-experimental storage duration was not identical for all specimens.

2.3. Tooth disinfection and canal preparation

The selected single-canal teeth underwent disinfection to ensure sterility. The teeth were immersed in 5.25% sodium

hypochlorite solution (Morvabon, Iran) for one hour to eliminate contaminants, followed by a thorough rinse with normal saline (Ghazi, Iran). Access cavities were then prepared using a long fissure bur, providing adequate access to the root canal. The working length of each canal was determined by inserting a K-File No. 15 (Dentsply, Switzerland) and observing the file's tip at the apical foramen. After marking the length, 1 mm was subtracted to establish the final working length. Root canal preparation was carried out using an Mtwo rotary file No. 35 with a 0.04 taper (VDW, Munich, Germany). During instrumentation, the canals were irrigated with 2 ml of 2.5% NaOCl between instruments to standardize chemical-mechanical preparation across all samples.

2.4. Medicament application

The samples were divided into two main groups of 64 teeth each. The control group did not receive any intracanal medicament. In the experimental group, calcium hydroxide paste was applied as a medicament. This paste was prepared by mixing calcium hydroxide powder (Golchadent, Iran) with normal saline in a 1.5:1 ratio. The paste was placed within 2 mm of the root apex using a Lentulo Spiral No. 25 (Micromega, France) attached to a low-speed handpiece (1000 rpm), and placement was confirmed via periapical radiographs. All access cavities were sealed with cotton and temporary filling material (Cavit G, 3M ESPE, Seefeld, Germany). Both groups were incubated at 37°C and 100% humidity for one week. After incubation, the calcium hydroxide paste was removed from the experimental group using EDTA and NaOCl irrigation combined with ultrasonic activation, along with mechanical cleaning using a K-File No. 30. The use of chelating and oxidizing irrigants with ultrasonic activation was intended to improve calcium hydroxide removal from the canal walls and dentinal tubules before obturation. The canals were then dried using paper cones (No. 35).

2.5. Sealer application and bond strength testing

Following canal preparation, each group was further divided into two subgroups of 32 teeth. Half of the samples in both the control and experimental groups were obturated with AH Plus sealer (Dentsply Detrey, Konstanz, Germany), while the other half were filled with Adseal sealer (Meta Biomed, Cheongju, Korea). Lot numbers were not available in the laboratory records. Gutta-percha cones (No. 35, 4% taper) were used with the cold lateral condensation technique for root canal obturation. This technique was selected to standardize obturation across all specimens, to use the same master cone size in all groups, and to avoid heat-related effects on resin sealer setting. The samples were incubated at 37°C and 100% humidity for one week to ensure complete sealer setting. After incubation, the specimens were sectioned into 2 ± 0.01 mm-thick slices from the middle third of the root using a diamond disk (Buchler Ltd, Evanston, IL, USA) under water cooling. Two or three eligible slices were obtained from each tooth, depending on root length and dimensional suitability. Cross-sectional measurements were performed using a caliper with 0.01-mm accuracy. A total of 300 root slices derived from the 128 teeth met the predefined dimensional criteria and were included in the mechanical push-out test; however, slices from the same tooth were not treated as independent statistical units. Push-out bond strength was evaluated using a universal testing machine (Zwick Roell Z020, Ulm, Germany) at a crosshead speed of 1 mm/min. Force was applied in the apico-coronal direction using a stainless-

steel cylindrical plunger with a 1.1-mm diameter until bond failure occurred, and the maximum force (in Newtons) was recorded to calculate bond strength in megapascals (MPa). Figure 1 shows a summary of the study process.

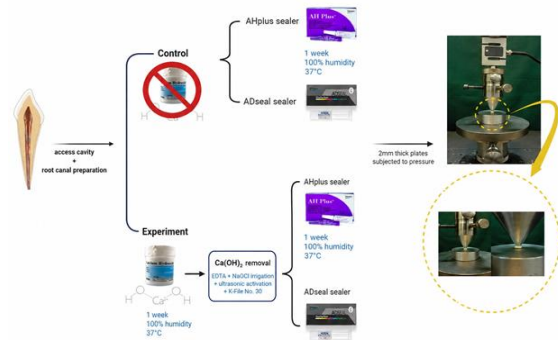


Fig. 1. The schematic process of evaluating the calcium hydroxide dressing effects on the bond strength of two resin-based endodontic sealers, AH Plus and Adseal.

2.6. Statistical analysis

The results were processed with SPSS Statistics software (SPSS for Windows, version 24, SPSS Inc., Chicago, IL, USA). The tooth was considered the experimental unit for statistical analysis. For teeth that provided more than one eligible slice, the push-out bond strength values of slices from the same tooth were averaged to generate a single tooth-level mean value. Therefore, statistical comparisons were based on 128 tooth-level observations ($n=32$ teeth/subgroup), not on 300 slice-level observations. Data normality was assessed using the Shapiro-Wilk test. Independent-samples t-tests were used to compare tooth-level bond strength values between groups. The results were considered significant at $p < 0.05$.

3. Results and Discussion

According to the inclusion and exclusion criteria, 128 teeth were included in the study. These 128 teeth were distributed into two control and experimental groups according to Table 1, and finally, four subgroups of 32 teeth were evaluated. A total of 300 eligible slices were obtained from these teeth for mechanical testing, and slice values from the same tooth were averaged for tooth-level statistical analysis.

The bonding strength of AH Plus and Adseal sealers was analyzed in two conditions: without calcium hydroxide (control group) and with calcium hydroxide dressing (experimental group). The reported values represent tooth-level mean bond strength values. The results are presented visually in Figure 2 for clarity.

In the control group, AH Plus demonstrated a higher bonding strength (34.28 ± 8.06 MPa) compared to Adseal (31.20 ± 6.84 MPa), with a statistically significant difference of 3.07 MPa in favor of AH Plus ($p = 0.01$). However, the presence of calcium hydroxide significantly altered the results. In the experimental group, the bonding strength of AH Plus decreased to 25.60 ± 10.18 MPa, while Adseal exhibited an increase to 32.39 ± 8.87 MPa, resulting in a significant difference of 6.79 MPa in favor of Adseal ($p < 0.001$).

Furthermore, the effect of calcium hydroxide on each sealer was evaluated. For AH Plus, calcium hydroxide caused a

significant reduction in bonding strength by 8.68 MPa ($p < 0.001$). In contrast, for Adseal, the presence of calcium hydroxide produced a small, statistically non-significant increase in bond strength from 31.20 ± 6.84 MPa to 32.39 ± 8.87 MPa (mean difference = 1.19 MPa, $p = 0.36$).

These findings, summarized in Figure 2, highlight the differential effects of calcium hydroxide on the bonding performance of these sealers. While AH Plus showed superior bonding strength in the control group, Adseal performed better in the experimental group, indicating that calcium hydroxide can influence the performance of sealers differently depending on their composition.

Table 1.
Studied groups.

No	Groups	Subgroups	Number
Group 1	Control group	AH plus sealer	32
		Adseal sealer	32
Group 2	Experimental group	AH plus sealer + Calcium hydroxide	32
		Adseal sealer + Calcium hydroxide	32

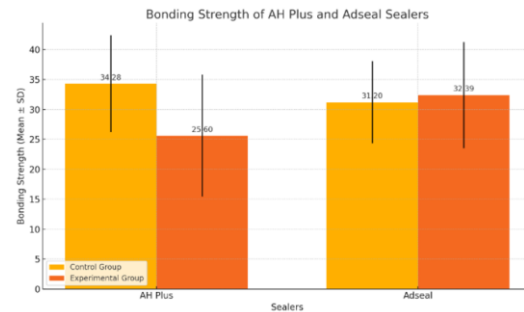


Fig. 2. Comparison of bonding strength of AH Plus and Adseal sealers in control (without calcium hydroxide) and experimental groups (with calcium hydroxide).

In this *in vitro* study, 128 single-rooted teeth were allocated into four experimental groups ($n = 32$ teeth/subgroup). Although 300 slices were mechanically tested, the tooth was used as the unit of analysis by averaging slice-level values obtained from the same tooth. Two groups were obturated with AH Plus or Adseal sealer without prior calcium hydroxide medication, whereas the remaining two groups received calcium hydroxide dressing before obturation with the respective sealers. The results demonstrated that AH Plus exhibited significantly higher push-out bond strength than Adseal in the absence of calcium hydroxide. In contrast, following calcium hydroxide medication, Adseal demonstrated significantly higher bond strength than AH Plus. Furthermore, calcium hydroxide significantly reduced the bond strength of AH Plus, whereas no significant effect was observed for Adseal. These findings suggest that AH Plus demonstrated superior bond strength in the absence of calcium hydroxide medication, whereas Adseal showed more favorable performance following calcium hydroxide treatment. However, further clinical studies are required before translating these findings into clinical recommendations.

From a materials-science perspective, both sealers evaluated in this study can be regarded as multicomponent dental compounds/composite systems. AH Plus contains an epoxy resin matrix with inorganic/radiopaque components, whereas Adseal contains an epoxy resin matrix combined with calcium phosphate and bismuth subcarbonate. Therefore, the present findings are relevant not only to endodontic adhesion but also to the behavior of resin-based dental compounds at the sealer-dentin interface, where the organic resin phase, inorganic filler phase, medicament residues, and dentinal substrate interact.

Root canal sealers play a crucial role in achieving an adequate seal between the filling material and dentinal walls, thereby contributing to the long-term success of endodontic treatment. The dislocation resistance of the sealer used in root canal treatment against tooth flexure, as well as the forces applied during preparation of the post space, are desirable goals in endodontic treatment [22]. Calcium hydroxide is the most common intracanal medicament used in the treatment of root canal infection, and substantial research has been performed on this material [23].

In this study, the average bonding strength of AH Plus sealer in the control group was significantly higher than the average bonding strength of Adseal sealer, and AH Plus showed better bonding performance than Adseal in the absence of calcium hydroxide. These findings are consistent with the studies of Guiotti et al. [24], Amin et al. [10], Carvalho et al. [25], and Ersahan et al. [22]. This superiority may be attributed to the ability of epoxy resin-based sealers to establish covalent bonds with exposed amino groups in dentinal collagen, resulting in enhanced adhesion and dimensional stability [24, 25].

After calcium hydroxide placement, the bond strength of AH Plus in the calcium hydroxide-treated group was significantly lower than that of the untreated control group. Similar findings were reported by Guiotti et al. [24], who related the significant reduction in AH Plus bond strength to blockage of dentinal tubules by residual calcium hydroxide and subsequent disruption of the sealer's micromechanical interaction with root dentin. Carvalho et al. reported that prior calcium hydroxide medication positively affected the bond strength of AH Plus sealer and suggested that the interaction between residual calcium hydroxide and the sealer may reduce dentinal permeability and contribute to improved sealing ability. In contrast, Amin et al. concluded that calcium hydroxide as an intracanal medicament had no significant effect on AH Plus, which may be attributable to differences in material brands, irrigation protocols, or experimental design [10, 25].

For Adseal, no significant difference was detected between the control group and the calcium hydroxide-treated group. This finding may be related to the chemical composition of Adseal, particularly the presence of calcium phosphate in its base paste. Residual calcium hydroxide may create a calcium-rich and alkaline interfacial environment. In a sealer containing calcium phosphate, this environment could theoretically favor calcium/phosphate interactions or apatite-like interfacial precipitation, thereby maintaining interfacial stability despite residual medicament. This mechanism was not directly tested in the present study and should be considered a hypothesis. Previous studies have shown that irrigation methods may not completely remove calcium hydroxide from the root canal [26, 27]. Hosoya et al. also reported that residual calcium hydroxide after removal and irrigation may increase the bonding or sealing behavior of calcium hydroxide-based and resin-based sealers [28].

In the present study, EDTA and NaOCl irrigation combined with ultrasonic activation was used during calcium hydroxide removal. This protocol was selected because chelating/oxidizing irrigation and ultrasonic activation can improve removal of calcium hydroxide residues from canal walls and dentinal irregularities. Nevertheless, complete removal of calcium hydroxide cannot be guaranteed, particularly within dentinal tubules, isthmuses, and surface irregularities. Therefore, the observed differences in bond strength should be interpreted as the combined effect of prior calcium hydroxide medication, its removal protocol, and the subsequent interaction of resin-based sealer compounds with the treated dentin surface.

In the present study, similar to the studies conducted by Amin et al. and Guiotti et al., the samples treated with calcium hydroxide were kept in an incubator at 37°C and 100% humidity for 7 days because the minimum time required for calcium hydroxide to exert

its antimicrobial effect is approximately one week. After obturation of the root canal, all samples were again kept in an incubator at 37°C and 100% humidity for 7 days until sealer setting. Incubation was performed to simulate intraoral temperature and humidity conditions, as described in previous studies [10, 24].

Similar to previous studies, the push-out test was used to evaluate the bond strength of endodontic sealers in the root canal. A known disadvantage of this method is the non-uniform distribution of shear stress and the deformation of gutta-percha in response to the compressive force applied during testing [22, 29]. In the present study, to reduce this problem, root canals inside the root fragments were standardized to improve contact between the tested endodontic sealers and the dentinal surface of the root canal [24]. The use of cold lateral condensation should also be considered when interpreting these findings. Lateral condensation was selected to create a standardized obturation procedure across all experimental groups and to avoid heat-related changes in the polymerization or flow of the resin-based sealers. Nevertheless, this technique applies lateral forces to the gutta-percha/sealer complex, and such horizontal pressure may affect the sealer-dentin interface differently from single-cone or warm vertical compaction techniques. Future studies should compare obturation techniques to determine whether the interaction between calcium hydroxide residues and resin-based sealer compounds is technique-dependent. The present study has some limitations. First, it was conducted under laboratory conditions that may not fully replicate the clinical environment. Second, because the extracted teeth were collected from different sources, the pre-experimental storage duration was not uniform across all specimens. Although all teeth were stored in normal saline until use to preserve dentin hydration, variations in storage time may have influenced dentin properties and push-out bond strength values. Third, residual calcium hydroxide was not quantitatively assessed using imaging techniques such as scanning electron microscopy or micro-computed tomography; therefore, the amount of residual medicament after EDTA/NaOCl irrigation with ultrasonic activation could not be directly verified. Fourth, failure mode analysis was not performed. Fifth, lot numbers of materials were not available in the laboratory records. Sixth, lateral condensation may influence the sealer-dentin interface differently from other obturation techniques. Therefore, caution should be exercised when extrapolating these findings to clinical practice.

4. Conclusion

Within the limitations of this *in vitro* study, AH Plus demonstrated significantly higher push-out bond strength than Adseal in the absence of calcium hydroxide medication. However, following calcium hydroxide treatment, Adseal exhibited significantly higher bond strength than AH Plus. Calcium hydroxide significantly reduced the bond strength of AH Plus, whereas no significant effect was observed for Adseal. These results suggest that resin-based endodontic sealers may respond differently to residual calcium hydroxide depending on their compound/composite formulation. Further laboratory and clinical studies are recommended to investigate the long-term clinical implications of these findings and to confirm their relevance under clinical conditions.

Ethical approval

This study was approved by Bushehr University of Medical Sciences with grant number 522 and by the Ethics Committee of Bushehr University of Medical Sciences

(IR.BPUMS.REC.1396.123). This study was conducted in Bushehr, Iran.

Author contributions

Nazanin Jafari: Conceptualization, Methodology, Data Curation, Writing—Original Draft Preparation, Writing—Review and Editing, **Mina Seyed Habashi:** Data collection, Writing—Original Draft Preparation, Writing—Review and Editing, **Maryam Khakpoor:** Data collection, Writing—Original Draft Preparation, Writing—Review and Editing, **Niusha Jafari:** Data collection, Writing—Original Draft Preparation, Writing—Review and Editing. All authors reviewed the manuscript and contributed to the critical reading and discussion of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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