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Research Article

Assessment of the Effect of Calcium Hydroxide and Double Antibiotic Paste as Root Canal Medications on the Bond Strength of AH Plus Root Canal Sealer: An *in Vitro* Study

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Abstract

Objective: The aim of the study was to evaluate and compare the Push-out bond strength of AH Plus root canal sealer after application of calcium hydroxide and double antibiotic paste as intracanal medications in single rooted extracted human teeth.

Methods: Thirty-six extracted human single rooted teeth were decoronated and prepared using Protaper universal rotary system to size F4. The roots were divided randomly into three groups according to the intracanal medication used.

Group I: Calcium hydroxide (CaOH), Group II: Double antibiotic paste (DAP) and Group III: Control group (No intracanal medication was placed). After three weeks, the intracanal medications were removed using the master apical file and 5 ml of 2.5% NaOCl irrigation. Then, final flush was done using 3 ml of 17% EDTA for 1 minute followed by 5 ml of saline solution. The root canals were obturated with gutta-percha and AH Plus sealer. Push-out test was applied to measure the bond strength of the sealer using universal testing machine.

Results: Concerning the push out bond strength values; DAP group showed the highest statistically significant mean push-out bond strength ($p = 0.006$). There was no statistically significant difference between the CaOH and the control groups. Concerning the mode of failure, there was no statistically significant difference between failure modes in the three groups.

Conclusion: The Bond strength of AH Plus sealer was affected by prior application of intracanal medicaments.

Keywords: AH Plus Sealer; Bond Strength; Calcium Hydroxide; Double Antibiotic Paste; Intra-Canal Medicaments

Introduction

Chemo mechanical preparation of the root canal space has an important goal of eliminating the intracanal bacterial population. However, it has been observed that even after irrigation, 40 - 60% of the root canal system still contains cultivable bacteria [1]. Thereby, intracanal medicaments have been used as an inter-appointment dressing to increase the incidence of bacteria-free canals [2].

For many years, Calcium hydroxide remains the most commonly used intracanal medicament because of its antimicrobial efficacy against most bacterial species identified in endodontic infections [3]. Since, root canal infections are regarded as polymicrobial, the antibiotic combinations have been suggested [4].

On the other hand, Gutta-percha is considered the most commonly used root canal filling material; however, it is criticized for its inability to bond to dentine wall causing inadequate seal of the root canal space and bacterial leakage to periapical area. So, the most widely accepted obturation technique is to use an adhesive root canal sealer in conjunction with gutta-percha [5]. Epoxy resin–based sealer (AH Plus; Dentsply DeTrey GmbH, Konstanz, Germany) is one of the most advocated used sealer due to its high bond strength to dentin wall [6]. However, It was shown that incomplete removal of the intracanal medications from the root canals would affect the adhesion of endodontic sealers to the root canal dentinal walls [7,8]. There are limited data available in the literature about the effect of the remaining intracanal medicaments on the bonding properties of epoxy resin-based sealer.

Thus, the purpose of this study was to assess the effect of intracanal medicaments on the bond strength of AH plus sealer to root canal dentin wall. The null hypothesis was that there would be no statistically significant difference between the effect of double antibiotic paste intracanal medicament and calcium hydroxide intracanal medicament on the bond strength of AH plus sealer.

Materials and Methods

Sample size

Based on previous study by Akcay., *et al.* (2014) [8] a total sample size of thirty-six teeth (12 in each group) were sufficient to detect a power of 80% and a significance level of 5%. The sample size was calculated by the G power program (Universidad Düsseldorf, Düsseldorf, Germany).

Selection of the samples

Thirty-six human permanent extracted single rooted teeth were selected for this study. Teeth were collected from the outpatient clinic of Oral Surgery Department, Faculty of Dentistry, Cairo University. These teeth were extracted due to periodontal reasons.

Eligibility criteria

Teeth with completely formed roots and having a well-developed apical constriction and with straight canals. Teeth with no internal or external root resorption and no signs of cracks. Preoperative radiographs were taken for all the teeth in both bucco-lingual and mesio-distal directions.

Preparation of the samples

The extracted teeth were immersed in 5.25% sodium hypochlorite (Clorox, Cairo, Egypt) for 30 minutes to remove any soft tissue remnants on the teeth surfaces. The teeth were cleaned from calculus and any other debris with ultrasonic scaler and then the teeth were stored in distilled water until use. The crowns of the teeth were removed at cementoenamel junction by using a water cooled double sided disc rotating in a low-speed handpiece to obtain a standardized root length of 15 mm. The canal patency was confirmed by inserting K-file size #10 (Mani, Tochigi, Japan). The working length was established by inserting K-file size #15 until it reached the apical foramen, then subtracting 1 mm from this measurement. All teeth were instrumented using Pro Taper universal rotary system (Dentsply Maillefer, Ballaigues, Switzerland). The master apical file was set as F4 for all the samples. The root canals were irrigated using 3 mL of 2.5% sodium hypochlorite between each file during instrumentation using a 27-gauge needle 1mm shorter than the working length, then 3 mL saline solution was used after NaOCl irrigation and 3 mL of 17% EDTA was used for 1 minute to remove the smear layer. Finally, the canals were flushed with 3 ml saline solution and dried with sterile absorbent paper points (Meta Biomed Co, Ltd, Korea).

Grouping of the samples

The thirty six specimens were randomly divided into three groups:

Group I (n = 12): Calcium hydroxide (CaOH) intracanal medicament (Dharma research Inc, Miami, USA) was applied. CaOH was prepared by mixing CaOH powder with distilled water with powder/liquid ratios of 3:1.

Group II (n = 12): Double antibiotic paste (DAP) was applied. DAP was prepared by mixing equal amounts of Metronidazole (Flagyl 500 mg tablets; Sanofi Aventis, El Amiriya, Egypt) and Ciprofloxacin (Cipro 500 mg tablets; European Egyptian Pharm, Alexandria, Egypt) with distilled water with powder/liquid ratios of 3:1.

Group III (n = 12): Control group where the root canals were obturated without previous application of intracanal medicaments.

The prepared pastes were placed into the root canals using a size #35 Lentulo spiral (Mani, Tochigi, Japan). The coronal opening of the root canals was sealed with a small cotton pellet and temporary filling material (3M ESPE, Seefeld, Germany) to avoid leakage. The specimens were stored at 37º C in 100% humidity for 3 weeks to simulate clinical conditions.

Obturation of the sample

After 3 weeks, the intracanal medicaments in groups I and II were removed using the master apical file F4 Portmapper rotary file and 5 mL of 2.5% NaOCl irrigation then 3 mL saline solution was used and then, the final flush was done using 3 mL of 17% EDTA for 1 minute followed by 5 mL of saline solution until there was no debris. Then, the root canals were dried using paper points size #40. The root canals were then obturated in lateral compaction technique using F4 as a master cone and auxiliaries of size #25/0.02. AH plus sealer (Dentsply, Konstanz, Germany) was mixed according to the instructions of the manufacturer. Radiographs with different shifts were taken to confirm the quality of the filling. All the teeth were stored at 37º C in 100% humidity for one week to allow the sealer to completely set before testing.

Bond strength evaluation

The bond strength of the sealer was measured by using the Push-out bond strength test. Each root was embedded in acrylic resin mold, that had a diameter of 2 cm, height of 3 cm and 0.5 cm thickness of wall. After setting of the acrylic resin, three horizontal sections of 2-mm thickness were obtained from coronal, middle and apical thirds of each root by using a low speed Water-cooled precision saw Iso Met 4000. The thickness of each slice was measured by using a digital calliper, which resulted in 36 horizontal sections per group. Each section was marked on its coronal side by using a marker. Each aspect of all the specimens was examined under stereomicroscope before testing to determine the diameter of the plunger to be used for Push-out test. Each root slice was placed in a loading fixture to avoid the movement of the samples during testing to ensure uniform stress distribution.

Each specimen was then subjected to a compressive load via a universal testing machine (Instron Corporation, Norwood, England) at crosshead speed of 0.5mm/min. Load was applied by three stainless steel cylindrical plungers of different sizes (0.5, 0.7 and 0.9 mm) that provided the most extended coverage over the filling material. The plunger tip was positioned so that it only contacted the filling material without touching the canal wall. The push-out force was applied in an apico-coronal direction until bond failure occurred, which was manifested by extrusion of the obturation material and a sudden drop along the load deflection. The force was recorded by using Bluehill computer software program.

The maximum failure load was recorded in Newton (N) and was used to calculate the push-out bond strength in Mega Pascals (MPa) according to the following formula.

Push-out bond strength (Mpa) = maximum load (N)/adhesion area of root canal filling (mm^2)

The adhesion (bonding) surface area of each section was calculated as:

$(\pi r^1 + \pi r^2) \times L$

L was calculated as:

$(r^1 - r^2)^2 + h^2$

Where π is the constant 3.14, r1 is the smaller radius, r2 is the larger and h is the thickness of the section in mm.

Failure mode analysis

Failure mode was investigated on both sides of the slices under a stereomicroscope at 50X magnification where the total extrusion of the material out of the specimen indicated that failure occurred.

The failure modes were classified as follows:

- Adhesive failure: Where, the sealer was totally separated from the Dentin (Dentin surface without sealer), it had two types:
  1. At root canal sealer-Dentin or.
  2. At root canal sealer-core.
- Cohesive failure: Occurs within the root canal sealer (Dentin surface totally covered with sealer).
- Mixed failure: It is a combination of adhesive and cohesive modes (Dentin surface partially covered with sealer).

Statistical methods

Numerical data were explored for normality by checking the distribution of data and using tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). Data showed non-normal (non-parametric) distribution. Data were presented as mean, median, standard deviation (SD), minimum, and maximum values. Kruskal-Wallis test was used to compare between the three groups. Friedman's test was used to compare between root levels. Dunn's test was used for pair-wise comparisons. Failure mode data (Qualitative data) were presented as frequencies and percentages. Chi-square test was used to compare between the three groups. The significance level was set at $P \le 0.05$. Statistical analysis was performed with IBM SPSS Statistics Version 20 for Windows.

Results

Push-out bond strength

Effect of different groups on the push-out bond strength:

In all groups; there was no statistically significant difference between mean Push-out bond strength values at the apical, middle and coronal root levels. ($P \ge 0.05$) (Table 1 and Figure 1).

Group	Apical		Middle		Coronal		P-value
	Mean	SD	Mean	SD	Mean	SD	
CaOH	5.80	2.00	4.38	1.32	4.60	2.56	0.368
DAP	7.56	2.69	6.38	1.60	6.23	2.22	0.307
Control	5.07	1.95	4.20	2.14	4.85	1.43	0.558

Table 1: Mean, standard deviation (SD) values and results of Friedman's test for comparison between Push-out bond strength at different root levels within each group.

*: Significant at $P \le 0.05$

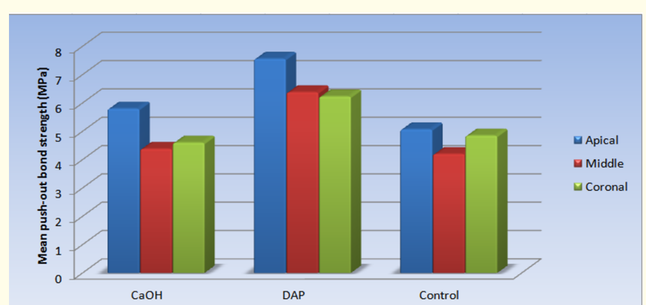

Figure 1: Bar chart representing mean Push-out bond strength at different root levels within each group.

Evaluation of the push-out bond strength test at different root levels

At the apical and coronal root levels; there was no statistically significant difference between the three groups.

At the middle root level; DAP showed the statistically significantly highest mean push-out bond strength. There was no statistically significant difference between CaOH and control groups; both showed statistically significantly lower mean push-out bond strength.

As regards the total push-out bond strength value; DAP showed the statistically significantly highest mean push-out bond strength. There was no statistically significant difference between CaOH and control.

Root level	CaOH (n = 12)		DAP (n = 12)		Control (n = 12)		*P*-value
	Mean	SD	Mean	SD	Mean	SD	
Apical	5.80	2.00	7.56	2.69	5.07	1.95	0.077
Middle	4.38 ^{B}	1.32	6.38 ^{A}	1.60	4.20 ^{B}	2.14	0.014*
Coronal	4.60	2.56	6.23	2.22	4.85	1.43	0.148

Table 2: Mean, standard deviation (SD) values and results of Kruskal-Wallis test for comparison between Push-out bond strength of the three groups.

*: Significant at P ≤ 0.05

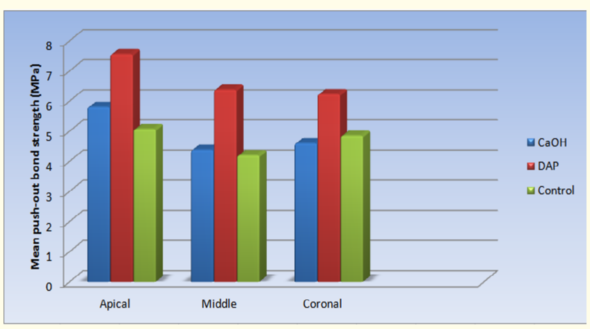

Figure 2: Bar chart representing mean Push-out bond strength of the three groups.

Mode of failure

Effect of different groups regarding the mode of failure

In all groups; there was no statistically significant difference between the different types of failure modes at the coronal, middle and apical root levels (Table 3 and Figure 3).

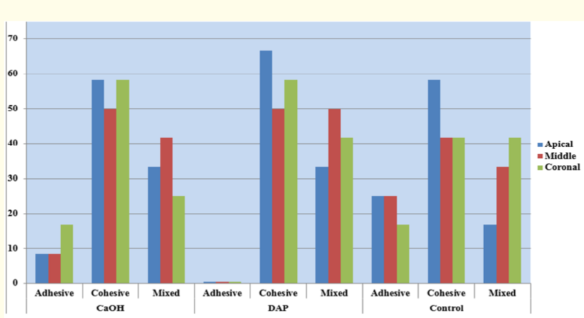

Figure 3: Bar chart representing failure modes at different root levels within each group.

No.		Apical		Middle		Coronal		P value
		%	No.	%	No.	%		
CaOH	Adhesive	1	8.3%	1	8.3%	2	16.7%	0.894
	Cohesive	7	58.3%	6	50.0%	7	58.3%	
	Mixed	4	33.3%	5	41.7%	3	25.0%	
DAP	Adhesive	0	0.0%	0	0.0%	0	0.0%	0.953
	Cohesive	8	66.7%	6	50.0%	7	58.3%	
	Mixed	4	33.3%	6	50.0%	5	41.7%	
Control	Adhesive	3	25.0%	3	25.0%	2	16.7%	0.737
	Cohesive	7	58.3%	5	41.7%	5	41.7%	
	Mixed	2	16.7%	4	33.3%	5	41.7%	

Table 3: Frequencies, percentages and results of Friedman's test for comparison between failure modes at different root levels within each group.

*: Significant at P ≤ 0.05

Evaluation of the mode of failure at different root levels

At all root levels; there was no statistically significant difference between failure modes in the three groups.

		CaOH (No. = 12)		DAP (No. = 12)		Control (No. = 12)		P value
		No.	%	No.	%	No.	%	
Apical	Adhesive	1	8.3%	0	0.0%	3	25.0%	0.355
	Cohesive	7	58.3%	8	66.7%	7	58.3%	
	Mixed	4	33.3%	4	33.3%	2	16.7%	
Middle	Adhesive	1	8.3%	0	0.0%	3	25.0%	0.403
	Cohesive	6	50.0%	6	50.0%	5	41.7%	
	Mixed	5	41.7%	6	50.0%	4	33.3%	
Coronal	Adhesive	2	16.7%	0	0.0%	2	16.7%	0.551
	Cohesive	7	58.3%	7	58.3%	5	41.7%	
	Mixed	3	25.0%	5	41.7%	5	41.7%	

Table 4: Frequencies, percentages and results of Chi-square test for comparison between failure modes in the three groups.

Significant at P ≤ 0.05.

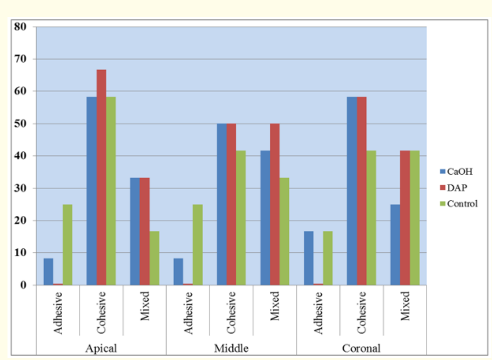

Figure 4: Bar chart representing the failure modes in the three groups.

Discussion

Successful endodontic treatment is an integrated process of proper cleaning, shaping and obturation. During biomechanical preparation, the use of intracanal medication is considered an important adjunct to eliminate different bacterial strains [9] since, irritants alone cannot achieve complete eradication of bacteria, CaOH has been the most commonly used intracanal medicament, owing to its high PH value, anti-inflammatory action and superior antimicrobial activity. However, it does not act efficiently against the resistant bacterial strains specially *Entrococcus Feacalis* presented in the endodontic infections [10]. Consequently, other materials were proposed as alternatives. Since introduced by Hoshino., *et al* [11], Triple antibiotic paste (TAP) has shown to have antibacterial activity [12,13]. It is composed of ciprofloxacin, metronidazole, and minocycline [11]. However, some studies reported that the minocycline component causes visible crown discoloration [2,14,15]. thereby, DAP was selected for that study. Previous studies showed that intracanal medicaments cannot be completely removed from the root canal walls using existing irrigation techniques [16,17].

In the present study 2.5% sodium hypochlorite and Protaper F4 rotary file followed 17% EDTA were used to remove the intracanal medicaments [18,19]. Where any remnant of intracanal medicaments may cause alteration in the chemical and structural composition of root canal dentin which may affect the chemical adhesion and penetration properties of endodontic materials [8,20].

AH plus endodontic sealer is considered the gold standard against which other sealers were compared owing to its low solubility, high flow rate, longer setting time and low volumetric polymerization shrinkage [21]. The relatively good adhesion of AH Plus sealer is believed to be attributed to the ability of an open epoxide ring to form a covalent bond with exposed amino groups of dentine collagen network [22].

Since many studies showed that smear layer removal before filling the root canal system enhanced the adhesion of endodontic sealers through increasing its ability to enter the dentinal tubules causing mechanical interlocking [23,24]. That's why we used 17% EDTA for smear layer removal.

The push-out test was selected as it measures the shear stress at the interface between dentine and cement which is comparable to the stresses under clinical conditions. Also it is effective, reproducible and less sensitive to small variations in stress distribution during load application [25]. In our procedures, the roots were cut into three slices (coronal, middle and apical) under water spray cooling system to avoid the heating effect during cutting that might affect the gutta-percha resulting in errors in the bond strength testing. Slicing of the roots for the push out test was made 2 mm thick with Isomet saw to permit adequate thickness for obturating materials and to prevent premature debonding during slicing [26].The thickness of each slice was measured by using a digital calliper to exclude the influence of specimen thickness variation. This thickness was used to be equivalent to the diameter of the plunger used in the push out test. The plunger tip was sized and positioned to touch only the filling material, without contacting the canal walls [25].

In the current study, our results showed that the highest statistically significant mean value of the bond strength was found in the DAP group when compared to the CaOH and the control groups. This result was in accordance to Gokturk., *et al.* 2016 [20] who reported that DAP improved the bond strength of AH plus. However, the lowest bond strength value in the DAP group was observed in the coronal root level compared to the apical and the middle root levels, this finding could be attributed to the large anatomical root canal space at the coronal third. Thus, higher circulation volume are allowed which facilitates the removal of the intracanal medicaments [27].

Our results were in agreement with Carvalho., *et al.* 2013 [28] and Gokturk., *et al.* 2016 [20] in terms of CaOH being able to improve the bond strength value of AH plus sealer without being statistically significant from the control group. Though, Amin., *et al.* 2012 [29], Ustun., *et al.* 2013 [30] and Akcay., *et al.* 2014 [8] stated that the use of CaOH didn't affect the bond strength of AH plus sealer. The conflict between the studies may resulted from different methodological variations such as single cone obturation technique [8,29], different intracanal removal techniques [29], period of storage after application of intracanal medicaments [30], different thickness of the root section [8] and using one plunger size during testing [29].

Though, there was no statistically significant difference between the mean push-out bond strength values at the different root levels, the highest bond strength values were found in the apical regions. Those results could be either related to the deeper sealer penetrations because of the high lateral condensation forces in the apical third or as a result of the marked variations in the structure along the root canal thirds, including irregular secondary dentine, accessory root canals and lack of tubules in the apical part of roots which increase the surface area of adhesion [31,32].

Since the adhesion of endodontic sealers to root canal dentin depends on the anatomy of the dentinal tubules and on the collagen fibres present, one possible reason for the middle root section in our study to show statistically significant difference between the groups is that the dentinal tubules and collagen fibers in the middle root third are more homogeneously distributed than in other root thirds. Also, the matching of the canal taper with the used gutta-percha might have proper fitting of the middle third to the prepared canal, leading to the uniform spreading of the sealer and a good bonding [33].

The mechanisms behind the different responses to DAP and CaOH may be due to the ability to remove them after treatment and their interactions with the dentin. Berkhoff., *et al.* 2014 [17] used different irrigation procedures to investigate the removal of Calcium hydroxide and antibiotic paste intracanal medicaments. They reported that the antibiotic paste appeared to have high diffusion and retention within the dentin with more than 80% of the paste being retained in the root canal regardless of the type of irrigation method used to remove it. On the other hand, most of the Calcium hydroxide was adequately removed.

The present study revealed that the mode of failure was mainly cohesive in all groups with no statistically significant among them. This result was in agreement with Ustun., *et al.* 2013 [30]. Similarly Shivanna and Bhargavi 2015 [27] reported that adhesive failure was the least observed failure mode for all groups.

In disagreement with these results, Akcay., *et al.* 2014 [8] showed no significant differences in the failure mode within the groups, whereas adhesive failure was the most frequent type of failure mode in all groups. Similarly Gokturk., *et al.* 2016 [20] reported that the most common type of failure was the adhesive failure. In this study, the null hypothesis that there was no influence of intracanal medicaments on the bond strength of the AH plus sealer was rejected.

Conclusion

Within the limitations of this study, it was concluded that application of intracanal medicaments affects the bond strength of AH plus sealer. DAP shows superior performance when compared to CaOH intracanal medicament.

Conflict of Interest

The authors deny any conflicts of interest in this study.

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