ORIGINAL RESEARCH

The Effect of Adding Various Antibiotics on the Push-out Bond Strength of a Resin-based Sealer: An *In Vitro* Study

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Abstract

Aim: This study aimed to compare the bond strength of AH Plus sealer to root canal dentin when used with or without various antibiotics including amoxicillin, clindamycin, and triple antibiotic mixture (TAM).

Materials and methods: A total of 80 single-rooted extracted human teeth were instrumented and obturated with gutta-percha and four different sealer-antibiotic combinations ($n = 20$). Group I: AH Plus without any antibiotics, Group II: AH Plus with amoxicillin, Group III: AH Plus with clindamycin, and Group IV: AH Plus with TAM. After seven days, the roots were sectioned perpendicular to their long axis and 1 mm thick slices were obtained from the midroots. The specimens were subjected to a push-out bond strength test and failure modes were also evaluated. Data was analyzed using Kruskal-Wallis and Dunn's *post hoc* tests.

Results: Group IV had significantly higher bond strength compared to other groups ($p \leq 0.05$). No significant differences were found between other groups. While the sealer-antibiotic groups predominantly showed cohesive failure modes, the control group displayed both cohesive and mixed failure modes.

Conclusion: Within the limitations of this study, the addition of TAM increased the push-out bond strength of AH Plus.

Clinical significance: Amoxicillin, clindamycin, or TAM can be added to AH Plus for increased antibacterial efficacy without concern about their effects on the bond strength of the sealer.

Keywords: AH Plus, Amoxicillin, Bond strength, Clindamycin, Triple antibiotic mixture.

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Introduction

Microorganisms play an essential role in the initiation and maintenance of pulpal and periapical diseases.[1,2] Therefore, the outcome of endodontic treatment depends on the eradication of bacteria and their byproducts during root canal treatment procedures.[3,4]

The presence of residual microorganisms after endodontic therapy or reinfection of a previously disinfected root canal environment is the main causes of endodontic treatment failure.[5,6]

Complete obturation of the root canal system is necessary to prevent coronal leakage and also to entomb the residual bacteria.[7,8]

To achieve a hermetic seal, the use of a sealer that firmly adheres to both root dentin and gutta-percha is essential.[9] Moreover, sealers should have antibacterial properties to prevent new bacterial growth and, ideally, maintain this effect over time.[10]

Resin–based sealers are widely used because of the lack of solubility and dimensional stability after setting.[11] However, most of these sealers exhibit some antibacterial activity only before setting and lose this ability as the materials set.[11,12]

The addition of different antibiotics has been suggested to enhance the antibacterial efficacy of endodontic sealers. Some studies have reported that adding amoxicillin increased the antibacterial efficacy of different sealers.[13–16] Similar results have been obtained when clindamycin was added to Kerr sealer.[13] Triple antibiotic mixture (TAM), which is a combination of ciprofloxacin, metronidazole, and minocycline, was also found to significantly increase the bactericidal properties of different sealers.[16,17]

It should be mentioned that amoxicillin and clindamycin are the antibiotics of choice for the treatment of endodontic infections and TAM is an antimicrobial intracanal medicament.[18,19]

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Although the addition of antibiotics to a sealer might improve its disinfecting properties, the changes in the physical properties must be kept within the standard limits.

Adhesion of sealers to root canal dentin is a required property for endodontic sealers. Although standard organization does not provide any data concerning the minimal required bond strength for endodontic sealers, it has been the subject of several studies.[20–22]

There are two reasons for the importance of assessing the bond strength of endodontic sealers to the dentin. First, improved seal can be expected with better adhesion of the sealer to the root canal. Second, better sealer adhesion can prevent the displacement of the obturation material during further restoration.[23,24]

To the best of our knowledge, the only published study concerning the effect of adding antibiotics on the bond strength of sealers concluded that the push-out bond strength of different sealers is not adversely affected by amoxicillin.[25]

However, there is limited data regarding the effects of adding other antibiotics on the adhesion of endodontic sealers to the root dentin. Therefore, this *in vitro* study sought to evaluate and compare the effects of adding TAM and two other antibiotics (amoxicillin and clindamycin) on the push-out bond strength of AH Plus sealer to root dentin.

Materials and Methods

The sample size for the push-out bond strength test was 80 at 95% power and a significance level of 0.05 using data (effect size = 1.18) obtained from a previous study.[26]

It is necessary to indicate that the present study was in accordance with the ethical standards of our Institutional Research Committee and the ethical code IR.SUMS.REC.1397.569 was obtained.

Sample Preparation

Eighty single-rooted extracted human teeth were used in this *in vitro* study. All teeth were disinfected by complete immersion in 2.5% sodium hypochlorite (Bonejeh, SBI, Iran) for 6 hours and then kept in normal saline (Darou Pakhsh, Tehran, Iran). After being thoroughly cleaned by removing the soft tissue and calculus from the root surfaces, the teeth were evaluated radiographically in mesiodistal and buccolingual directions. The teeth with calcified canals, internal/external resorption, extra canals, previous root canal treatment, immature root apices, and root curvature more than 10° were excluded from the study.

Using a high-speed carbide bur and water spray, the specimens were decoronated to acquire a standardized root length of 15 mm. A size 15 K-file (Mani, Japan) was used to establish patency and determine the working length. The teeth were prepared using ProTaper rotary (Dentsply, Maillefer) instruments up to size F3 (size 35, 0.06 taper) according to the manufacturer's instructions.

The root canals were irrigated with 2 mL 5.25% sodium hypochlorite between the instruments. They were finally rinsed with 5 mL 17% ethylene diamine tetra acetic acid (EDTA) for 1 minute to remove the smear layer.

After drying the root canals with paper points, the specimens were randomly divided into four experimental groups ($n = 20$ each) and different antibiotics were added to the AH Plus sealer (Dentsply, DeTrey, Germany).

Group I (control): AH Plus without adding any antibiotics

Group II: AH Plus + Amoxicillin (Farabi, Pharmaceutical, Iran)

Group III: AH Plus + Clindamycin (Soha, Iran)

Group IV: AH Plus + Triple antibiotic powder

Amoxicillin and clindamycin powder were obtained by emptying commercially available capsules. In order to prepare TAM powder, minocycline (Hexal, Germany), ciprofloxacin (Rouz Darou, Iran), and metronidazole (Fars Darou, Iran) tablets were crushed using a clean mortar and pestle. The AH Plus sealer was then weighed and mixed with antibiotic powders at 10% of the sealer's total weight.

All teeth were obturated using a single-cone technique by matching F3 taper gutta-percha cones (Cheongju Factory, Korea). For this purpose, a Lentulo spiral filler 30 (Mani, Japan) was used to fill the root canals with the sealers explained above. Then, a single gutta-percha cone was slightly coated with the sealer and placed in the root canal to the working length.

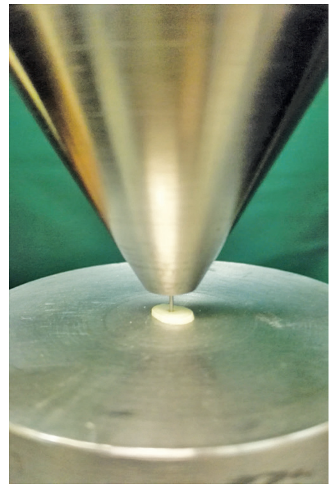

Fig. 1: Push-out bond strength test

Radiographs were taken to confirm complete filling. After coronal sealing with a glass ionometer (GC Corporation, Tokyo, Japan), the obturated teeth were incubated at 37°C and 100% humidity for 14 days to allow the sealer to be completely set. The specimens were ultimately sectioned perpendicular to their long axis using a slow-speed saw (Mecatome T180, Presi SA, Angonnes, France) under water cooling. One slice with approximately 1 + 0.1 mm thickness was obtained from the midroot of each tooth. Therefore 20 disks in each group were subjected to push-out test. A digital camera attached to a stereomicroscope was employed to capture images from the coronal and apical sides of the slices at 32 × magnification. ScopeImage software (Best Scope-3060c, China) was then used to measure the lumen diameters of both sides of the slices. The height of the slices was measured using a digital caliper (Mitutoyo, Tokyo, Japan).

Push-out Bond Strength Application

A plunger which most closely matched to the filling material and the diameter of the root canal was chosen and connected to a universal testing machine (ZwickRoell Group; Germany). An apico-coronal vertical load was applied to the obturation material at the crosshead speed rate of 0.5 mm/min and the maximum load before failure was recorded in newtons. The push-out bond strength in megapascals (MPa) was calculated as the maximum load divided by the adhesion area (Fig. 1).

The adhesion area of the root canal filling was calculated using the following formula:

$$A = \pi(r + R)[h^2 + (R - r)^2]^{0.5}$$

Where π is the constant 3.14, R and r represent the radii of the coronal and apical sides, respectively, and h is the thickness of the root slice.[27]

After the push-out test, each sample was visually examined under a stereomicroscope at 32 × magnification to determine the type of failure bond. Failure modes were classified as adhesive (at the dentin-sealer interface), cohesive (within the root canal sealer), and mixed (a combination of adhesive and cohesive failure modes).

Table 1: Effect of antibiotics on the push-out bond strength of AH Plus

Experimental group	*Number*	*Mean (Median) ± SD*	*Failure mode (A–C–M) %*
Group I (AH plus alone)	20	0.28 (0.25) ± 0.14[a]	0–48–52%
Group II (AH plus with amoxicillin)	20	0.36 (0.26) ± 0.38[a]	0–76–24%
Group III (AH plus with clindamycin)	20	0.26 (0.19) ± 0.25[a]	0–76–24%
Group IV (AH plus with TAM)	20	0.42 (0.39) ± 0.14[b]	0–92–8%

Different lowercase superscript letters in columns mean significant difference at $p = 0.05$ level. A, adhesive; C, cohesive; M, mixed

As the distribution of the data was not normal, the non-parametric Kruskal–Wallis test and Dunn's *post hoc* test were used to analyze the data. All statistical analyses were performed with SPSS (version 20, SPSS Inc, Chicago, IL, USA) at a significance level of 0.05.

Results

The mean, median, and standard deviation of the push-out bond strength values of the experimental groups are presented in Table 1. The experimental groups had a statistically significant difference in terms of push-out bond strength ($p < 0.001$). Pairwise comparisons showed that Group IV (AH Plus + TAM) had significantly higher bond strength compared to other groups ($p < 0.05$). No significant differences were found between other groups ($p > 0.05$).

The results of the mode of failure analysis are summarized in Table 1. Adhesive failure was not detected in any of the experimental groups. While the groups containing antibiotics predominantly showed cohesive failure modes, the control group displayed both cohesive and mixed failure modes.

Discussion

An ideal root canal sealer must adhere to the root canal dentin and resist dislocating forces.[28] The push-out bond strength used in the present study is a common test to evaluate the effectiveness of dental materials' adhesion to the root dentin.[29–31] Although recently the value of studies on dislodgement resistance of root fillings has been questioned, a correlation has been demonstrated between push-out bond strength and sealing ability of AH Plus.[32,33] Furthermore, the push-out test provides valuable information comparing different sealers or obturation techniques.[34]

AH Plus sealer is an epoxy resin-based sealer with good properties such as deep penetration into micro irregularities and expansion after water sorption which increases its resistance against dislodgement.[35] Although the manufacturer claims that AH Plus sealer has good antimicrobial effects, Zhang et al. reported that the antimicrobial effects of the fresh AH Plus sealer diminished after one day.[36] Adding antibiotics to a sealer can improve its antimicrobial effects over time and reduce the concentration of microbes to a level that can be eliminated by a favorable host response.[15] Based on this rationale, antibiotics have been added to different sealers and successfully enhanced their antibacterial properties.[13,16] However, antibiotics may influence the bond strength of the sealer to the root canal not only by changing the chemical properties of the sealers but also because of their direct effect on the dentinal walls. The results of the present study showed that adding TAM to AH Plus sealer (at a 1:10 ratio) improved the sealer's push-out bond strength. No previous study has evaluated the effect of adding TAM on the bond strength of endodontic sealers. However, our finding is in line with the results of Akcay et al. and Sobhnamayan et al. who reported that when used as an intracanal dressing, TAM improved the bond strength of AH to dentin.[27,37]

This finding can be attributed to the fact that minocycline in the TAM binds to calcium ions via chelation and forms an insoluble complex in the tooth matrix.[38] The results of the present study also showed that amoxicillin and clindamycin had no adverse effects on the push-out bond strength of the sealer. This finding is in accordance with that of Vemisetty et al.[25] who reported that the bond strength of different endodontic sealers was not affected by adding amoxicillin to the mixture. On the other hand, Andolfatto et al. reported that the incorporation of amoxicillin in the concentration of 10% significantly decreased the flow of AH Plus.[39] The significance of the flow of an endodontic sealer on its adhesion to the dentin however is not validated yet.

In failure pattern assessments, none of the experimental groups in the present study showed adhesive failure. While the groups containing antibiotics predominantly showed cohesive failure modes, the control group (without antibiotics) displayed both cohesive and mixed failure modes. This result is in agreement with the findings of Adl et al.[22] and Misgar et al.[40] who reported a combination of cohesive and mixed failure mode for AH Plus sealer.

Although our findings showed that the bond strength of the tested sealer was not adversely affected by adding different antibiotics, there are concerns about other physical properties of the sealers. Therefore, further studies are required to address other physical characteristics, like sealing ability, flowability, and solubility of the mixture of antibiotics and endodontic sealers.

Conclusion

Within the limitation of this *in vitro* study, the authors' conclusion supports the utilization of TAM as an effective enhancer for improving the bond strength of AH Plus sealer, thus recommending its usage. Moreover, adding amoxicillin and clindamycin had no adverse effects on the bond strength of this endodontic sealer.

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