

The Effect of Adding Different Antibiotics on the Resistance against Bacterial Leakage of AH 26 Sealer

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Abstract

Aim: Most endodontic sealers show antimicrobial activity before setting, but most of them also lose this ability after setting. Addition of an antibiotic may affect the properties of sealers such as sealing ability, setting time, and so on. The aim of this study was to assess whether the addition of antibiotics (amoxicillin, doxycycline, and clindamycin) improves the sealing ability of AH 26 sealer. **Materials and Methods:** Seventy extracted human mandibular premolars were used. After cleaning and shaping the canals, the teeth were divided into six groups: group 1: gutta-percha and AH 26 sealer, group 2: gutta-percha and AH 26 sealer+doxycycline, group 3: gutta-percha and AH 26 sealer+amoxicillin, group 4: gutta-percha and AH 26 sealer+clindamycin, group 5: gutta-percha without sealer (positive control), and group 6: gutta-percha and AH 26 sealer (the root surface were covered with nail varnish) (negative control). A microbial leakage model was used to assess the sealing ability. **Results:** Group 2 had the greatest resistance against bacterial leakage. Furthermore, combining AH 26 sealer with amoxicillin and clindamycin increased mean leakage time compared to AH 26 sealer solely. However, the differences between groups 1 and 3 as well as between groups 1 and 4 were not statistically significant. **Conclusion:** Incorporating antibiotics especially doxycycline into

AH 26 sealer increases its resistance against bacterial leakage.

Keywords: AH26 sealer, Antibiotics, Bacterial leakage, Enterococcus faecalis.

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Introduction

Following cleaning and shaping, root canal system must be filled three dimensionally with gutta-percha and sealer. The three major functions of root canal obturation include entombing remaining microorganisms within the root canal system, preventing the influx of periapical exudates from re-entering the root canal, and preventing coronal leakage (1). Furthermore, although considerable microbial reduction can be achieved by the biomechanical instrumentation, irrigation, and intracanal medication, studies have demonstrated the presence of bacteria in dentinal tubules and cementum after treatment (2). For this reason, and particularly when pulpal necrosis and apical periodontitis are present, the choice of a sealer with antimicrobial activity might help to decrease or avoid growth of the remaining microorganisms (3). Considering the fact that gutta-percha does not bond to dentin (4), a sealer is required to adhere gutta-percha to dentin as well as gutta-percha cones together (1). One of the properties of an optimal sealer is its antimicrobial activity.

Many endodontic sealers may show antimicrobial activity before complete setting (5, 6); however, most of them also lose this activity after setting. Therefore, few studies have suggested the incorporation of antibiotics in sealers (7, 8). However, the addition of an antibiotic may affect the properties of sealers such as sealing ability, setting time, diffusibility, and so on (8). To date, the effect of adding antibiotics on the sealing ability of sealers has not been reported. Therefore, the aim of the present study was to evaluate whether the addition of antibiotics (amoxicillin, doxycycline, and clindamycin) improves the sealing ability of AH 26 sealer.

Materials and Methods

Seventy extracted human mandibular premolars were used in this study. All of the selected teeth were caries-free and contained either no coronal (or minimal) restoration. All teeth possessed fully formed apices. The teeth had been stored in 10% formalin and were kept moist during the experiment period. The teeth were radiographed both from the buccal and proximal directions to ensure that one single straight canal was present.

A standard access cavity was prepared on each tooth using long fissure burs (Tizkavan, Tehran, Iran). Canal length was determined using #10 or #15 K-file (Kerr, Romulus, MI, USA) through the canal space until the tip could be seen to exit the apical foramen. Working length was determined by subtracting 1 mm from the canal length. Coronal part of the canals were flared with size #2 to #4 gates-glidden drills (Dentsply, Maillefer, Switzerland), and the apical part of the canal was

instrumented to # 30 using hand instruments. The canal was flushed with 2 ml of 2.6% sodium hypochlorite (Sigma Chemicals Co., St. Louis, MO, USA) between every instrument, and apical patency was maintained with a size 10 file throughout the instrumentation. Furthermore, 5 ml of 17% EDTA (pH: 7.2) rinses were used during and after instrumentation to remove the smear layer and decrease coronal leakage (7). The teeth were divided into 6 groups, 4 experimental groups of 15 teeth each and also 2 control groups of 5 teeth each as follows:

Group 1: Obturated with gutta-percha and AH 26 sealer

Group 2: Obturated with gutta-percha and AH 26 sealer+doxycycline

Group 3: Obturated with gutta-percha and AH 26 sealer+amoxicillin

Group 4: Obturated with gutta-percha and AH 26 sealer+clindamycin

Group 5: Obturated with gutta-percha without sealer (positive control)

Group 6: Obturated with gutta-percha and AH 26 sealer (all the root surfaces including apical foramen were covered with two layers of nail varnish) (negative control)

Three antibiotics including doxycycline (100mg capsules), amoxicillin (500mg capsules), and clindamycin (300mg capsules) (Darou Paksh, Tehran, Iran) were prepared with a mortar and pestal and added separately to AH 26 sealer (added to the mixed AH 26). The amount of antibiotic added was equivalent to 10% of the sealer's total weight. Antibiotics were mixed according to manufacturers' instructions. All canals were obturated using lateral compaction technique. A heated plugger was used to remove excess filling material until a standardized length of 10 mm of filling was left in each canal.

Teeth were stored in a piece of gauze that was dampened with Tryptic Soy Broth as storage media, enclosed in sealed tubes, and placed in incubator for 2 weeks at 37°C to allow complete setting. The microbial leakage model consisted of an upper and a lower chamber. Culture medium, 5 ml was placed in 70 individual test tubes. Each of the 70 teeth was placed into the test tube so that at least 2 mm of each apex was within the storage media. The chamber of each tooth was filled with *Enterococcus faecalis* suspension on day 0. A fresh bacterial suspension of *Enterococcus faecalis*, which was prepared daily, was added to the access opening of each tooth until the chamber was full. This stage was performed every day throughout the study. Penetration of the canal was recorded when turbidity was noted in the broth. Cultures were checked daily until the final test system became positive at day 90. Data were analyzed using Kruskal-Wallis.

Furthermore, Dunn's method was used to pairwise multiple comparisons between all groups. P-value < 0.05 was considered significant.

Results

All positive control teeth revealed rapid and consistent bacterial leakage within 24–48 hour, none of negative control teeth showed bacterial leakage throughout the experiment. The resistance against

bacterial leakage was significantly greater in group 2 comparing other groups (groups 1, 3, and 4) between groups 1-4 ($p < 0.05$) (Fig. 1). Combining AH 26 sealer with amoxicillin and clindamycin increased mean leakage time compared to AH 26 sealer solely. However, the differences between group 1 and 3 as well as between groups 1 and 4 were not statistically significant ($p > 0.05$).

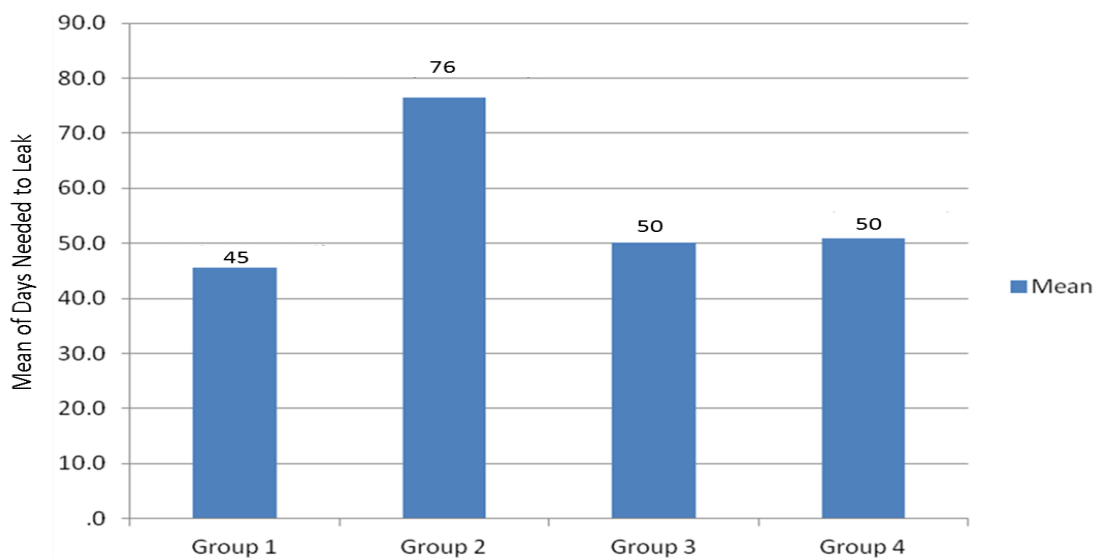


Figure 1. Mean of the number of days needed to leak in different experimental groups

Discussion

Microorganisms are considered to be the primary etiologic agents in pulp and periapical diseases (9-11). In this study, *Enterococcus faecalis* was selected for some reasons. It may be found in up to 40% of primary endodontic infections. However, its frequency in persistent periapical lesions has been indicated to be 9 times higher. Its prevalence in root-filled teeth with periapical lesions using polymerase chain reaction (PCR) is 67-77% (12). Its capacity to endure long periods of starvation until an adequate nutritional supply becomes accessible has been documented (13). Chemo-mechanical preparation, irrigation solutions and intracanal medicaments significantly reduce the intracanal microbial load (14). However, *ex vivo* and clinical documents has revealed that mechanical preparation leaves significant parts of the canal walls untouched and unbridged (15). Furthermore, the high surface tension of sodium hypochlorite, as a routine root canal irrigant, does not allow it to reach microorganisms in the depth of dentinal tubules (16).

Sealers play an essential role in preventing microbial leakage into the root canal system and in the

entombment of remaining microorganisms to the root canal system (17). Furthermore, most sealers have both antibacterial and cytotoxic effects (18), and these properties may limit the ingress of bacteria. In order to enhance the antibacterial activity of sealers, adding antibiotics has been suggested. Indeed, local application of antibiotics may be a better choice for delivering antibiotics comparing prescribing systemic antibiotics. This kind of application also may decrease the risk of allergic reactions and cytotoxicity (19).

In the present study, the effect of incorporating an antibiotic in AH 26 sealer was assessed. Findings showed that the incorporation of doxycycline into AH 26 sealer improved the sealing ability of root canal filling. It has been demonstrated that tetracyclines including doxycycline can attach to dentin and be released over time (the property called substantivity). Better sealing ability of combining AH 26 sealer compared to other groups can be attributed to the substantivity of doxycycline. Khademi et al. (20) revealed the substantivity of doxycycline for up to 4 weeks. In another study, Mohammadi and Shahriari (21) demonstrated the substantivity of MTAD (a doxycycline-based root canal irrigant) for up to 4

weeks. Another study showed the substantivity of Tetraclean (a doxycycline-based root canal irrigant) for up to 4 weeks (22). Limited studies have demonstrated that incorporating antibiotics in sealers increased the antibacterial activity of sealers. Using agar diffusion test, Hoelscher et al. (8) showed that adding antibiotics (amoxicillin, clindamycin, doxycycline, penicillin, and also metronidazole) may enhance the activity of sealer against *Enterococcus faecalis*. In another study using direct contact test, it was shown that mixing amoxicillin with sealers increased the efficacy of the sealers (7).

The reason for adding 10% antibiotics to AH 26 sealer was based on the study by Hoelscher et al. (8) who found that sealer-antibiotic combinations containing amoxicillin, penicillin, clindamycin, and doxycycline had a significant difference in the mean zones of inhibition when compared to Kerr EWT sealer alone.

AH Plus sealer is an epoxy resin-based sealer which has been used as reference in some leakage studies (23). In a bacterial leakage study using *Streptococcus mutans*, Eldeniz and Ørstavik (18) found that all the specimens filled with AH Plus sealer leaked within 13 days. They attributed this finding to the shrinkage of AH Plus sealer during the setting process, and/or as a result of diminished antibacterial activity of this sealer. In the present study, the mean leakage time of the AH 26 sealer group was 46 days and up to day 33 only 25% of the specimens were leaked. This difference between leakage times can be attributed to the type of sealer and the type of bacterium used. Although AH Plus and AH 26 have similar compositions, formaldehyde release from AH 26 may help killing the microorganisms. Another study using *Enterococcus faecalis* demonstrated that bacterial leakage occurred at the third day for AH Plus, the fourth day for Sealapex, and the seventh day for AH 26 and Ketac-Endo which is in contrast to the present findings (24).

Adding antibiotics to root canal sealers increases their antibacterial activity. Furthermore, it seems that incorporating doxycycline in AH 26 sealer increases its resistance against bacterial leakage. However, it should be kept in mind that antibiotics may change the setting time, shrinkage, diffusibility, and other properties of sealers. Further studies are needed to evaluate all these aspects.

Finally, it is necessary to mention limitations of the present study. It should be kept in mind that the present study was *in vitro*. Extrapolation of the present findings to clinical conditions should be done with caution. It should be noted that conducting bacterial leakage studies is impossible *in vivo*. However, one of the reliable methods to assess leakage *in vitro* is bacterial leakage. Furthermore, sensitivity of patients to

antibiotics especially penicillin and its derivatives should be kept in mind in clinical studies.

Conclusion

Incorporating antibiotics especially doxycycline to AH 26 sealer increase its resistance.

References

1. Orstavik D. Materials used for root canal obturation: technical, biological and clinical testing. *Endod Topics* 2005; 12: 25-38.
2. Sen BH, Piskin B, Demirci T. Observation of bacteria and fungi in infected root canals and dentinal tubules by SEM. *Endod Dent Traumatol* 1995; 11: 6-9.
3. Spangberg L, Engström B, Langeland K. Biologic effects of dental materials. 3. Toxicity and antimicrobial effect of endodontic antiseptics *in vitro*. *Oral Surg Oral Med Oral Pathol* 1973; 36: 856-871.
4. Aptekar A, Ginnan K. Comparative analysis of microleakage and seal for 2 obturation materials: Resilon/Epiphany and gutta-percha. *J Canadian Dent Assoc* 2006; 72: 245.
5. Abdulkader A, Duguid R, Saunders EM. The antimicrobial activity of endodontic sealers to anaerobic bacteria. *Int Endod J* 1996; 29: 280-283.
6. Mickel AK, Nguyen TH, Chogle S. Antimicrobial activity of endodontic sealers on *Enterococcus faecalis*. *J Endod* 2003; 29: 257-258.
7. Hoelscher AA, Bahcall KJ, Maki JS. *In vitro* evaluation of the antimicrobial effects of a root canal sealer-antibiotic

- combination against *Enterococcus faecalis*. *J Endod* 2006; 32: 145-147.
8. Baer J, Maki JS. In vitro evaluation of the antimicrobial effect of three endodontic sealers mixed with amoxicillin. *J Endod* 2010; 36: 1170-1173.
 9. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. *Oral Surg Oral Med Oral Pathol* 1965; 20: 340-349.
 10. Möller AJ, Fabricius L, Dahlén G, Öhman AE, Heyden G. Influence on periapical tissues of indigenous oral bacteria and necrotic pulp tissue in monkeys. *Scand J Dent Res* 1981; 89: 475-484.
 11. Sundqvist G. Ecology of the root canal flora. *J Endod* 1992; 18: 427-430.
 12. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. *Enterococcus faecalis*: its role in root canal treatment failure and current concepts in retreatment. *J Endod* 2006; 32: 93-98.
 13. Rôças IN, Siqueira JF, Santos KR. Association of *Enterococcus faecalis* with different forms of periradicular diseases. *J Endod* 2004; 30: 315-320.
 14. Mohammadi Z. Chemomechanical strategies to manage endodontic infections. *Dent Today* 2010; 29: 91-92.
 15. Peters OA, Laib A, Göhring TN, Barbakow F. Changes in root canal geometry after preparation assessed by high-resolution computed tomography. *J Endod* 2001; 27: 1-6.
 16. Giardino L, Ambu E, Becce C, Rimondini L, Morra M. Surface tension comparison of four common root canal irrigants and two new irrigants containing antibiotic. *J Endod* 2006; 32: 1091-1093.
 17. Mohammadi Z, Yazdizadeh M. Evaluation of the antibacterial activity of new root canal sealers. *J Clin Dent* 2007; 18: 70-72.
 18. Eldeniz AU, Orstavik D. A laboratory assessment of coronal bacterial leakage in root canals filled with new and conventional sealers. *Int Endod J* 2009; 42: 303-312.
 19. Mohammadi Z, Abbott PV. On the local applications of antibiotics and antibiotic-based agents in endodontics and dental traumatology. *Int Endod J* 2009; 42: 555-567.
 20. Khademi A, Mohammadi Z, Havaee A. Evaluation of the antibacterial substantivity of several intra-canal agents. *Aust Endod J* 2006; 32: 112-115.
 21. Mohammadi Z, Shahriari S. Residual antibacterial activity of chlorhexidine and MTAD in human root dentin in vitro. *J Oral Sci* 2008; 50: 63-67.
 22. Mohammadi Z, Giardino L, Mombeinipour A. Antibacterial substantivity of a new antibiotic-based endodontic irrigation solution. *Aust Endod J* 2012; 38: 26-30.

23. Brackett MG, Martin R, Sword J, et al.
Comparison of seal after obturation
techniques using a polydimethylsiloxane-
based root canal sealer. J Endod 2006;
32: 1188-1190.

24. Yucel AC, Guler E, Guler AU, Ertas E.
Bacterial penetration after obturation

with four different root canal sealers. J
Endod 2006; 32: 890-893.

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