Combined Bacterial-Fungal Penetration After Obturation with AH 26 and AH Plus Root Canal Sealers

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Abstract:
Objective: The aim of this in vitro study was to compare the coronal microleakage between Streptococcus sanguis per se and a mixture suspension of E. faecalis and C. albicans in root canals filled with Gutta-percha and either AH 26 or AH Plus sealer.

Materials and Methods: One-hundred and ten extracted human teeth were decoronated to a standardized root length of 14 mm and prepared using Mtwo nickel-titanium (Ni-Ti) instruments to a master apical file size 35, 0.04 taper and obturated with gutta-percha and either AH 26 or AH Plus sealers by lateral condensation. After setting of the sealers, the teeth were randomly divided into two experimental groups (n=50) and two control groups (n=5). The coronal chambers of half the teeth in each experimental group (n=25) were inoculated with 0.5 ml of Brain Heart Infusion agar containing approximately 3×10^8 of each microorganism in every ml of Candida albicans (ATCC10231) and Enterococcus faecalis (ATCC29212) using a sterile micropipette. The other half of the teeth of each experimental group were inoculated with Streptococcus sanguis (ATCC10556) with the same concentration. The days of microbial penetration were noted for evaluation. The data were statistically analyzed using Kaplan Meier and log-rank tests.

Results: There were no statistically significant differences between the four experimental groups regarding the leakage rate (P=0.130).

Conclusion: Under the conditions of this study, there was no difference in the microbial penetration of AH 26 and AH Plus sealers at 60 days.

Key Words: AH Plus; AH 26; Root Canal Filling Materials

INTRODUCTION
Two important objectives of root canal treatment are thorough removal of the microbial source of infection and filling root canals in three dimensions. The combination of gutta-percha and a sealer is the most commonly used filling material in clinical practice [1]. AH 26 and AH Plus are two epoxy resin-based sealers. Several studies have demonstrated the sufficient adhesion capacity of AH 26 to the root canal walls [2-7]. As described previously in literatures, AH Plus has improved properties, including long-term dimensional stability [8], shorter setting time and more radiopacity compared to AH 26 [9].

In some studies, coronal leakage has been considered as a major cause of persistent postoperative disease [10,11]. The sealing ability of the root canal sealer has been evaluated by various leakage markers such as dyes, radioisotopes, and bacteria. Because of inherent problems in dye and radioisotope markers, us-
ing bacteria in leakage studies may provide more biologically and clinically relevant information [12,13]. Enterococcus faecalis and Candida albicans have been isolated from infected root canals associated with periapical lesions [14-16]. Both microbes are determined as the most resistant microorganisms in endodontic infections and are considered as a possible cause of endodontic treatment failure [14]. Peciuliene et al [15] found Candida albicans in 18% of cases with chronic apical periodontitis always associated with other bacteria, and in 50% of the cases associated with Enterococcus faecalis. To date no published article has been found to compare the penetration of a combined bacterial-fungal suspension with a single bacterial strain. Therefore, this in vitro study was designed to compare coronal leakage of Streptococcus sanguis per se with a mixture suspension of E. faecalis and C. albicans in root canals filled with Gutta-percha and either AH 26 or AH Plus sealer.

**MATERIALS AND METHODS**

One-hundred and ten extracted human teeth with straight, single root canals stored in 0.1% thymol were selected for this study. The teeth were decoronated to a standardized root length of 14 mm. The working length was measured by deducting 1 mm from the length recorded when tip of a #15 file (Dentsply Maillefer, Tulsa, USA) was visible at the apical foramen. The specimens were prepared using Mtwo Ni-Ti instruments (VDW, Munich, Germany) to a size 35, 0.04 taper master apical file and were irrigated between files with 2 ml of 5.25% sodium hypochlorite. The coronal part of the root canals was shaped using size 2 and size 3 Gates-Glidden drills (Dentsply, Maillefer, Ballaigues, Switzerland). Apical patency was confirmed by inserting a #15 file through the apical foramen before and after root-canal preparation.

After instrumentation, the smear layer was removed with 17% EDTA followed by 5.25% NaOCl, and the canals were dried with sterile paper points. The teeth were then randomly divided into two experimental groups (n=50) and obturated by lateral condensation of gutta-percha (Gapadent Co. LTD, Tianjin, China) and sealer. In the first group, AH 26 silver-free (Detrey Dentsply, Germany) was used as the sealer, and AH Plus (Detrey Dentsply, Germany) was used in the second group. The sealers were mixed according to the manufacturer’s instructions and applied to the walls of the canal with a size D finger spreader (Dentsply, Maillefer, Ballaigues, Switzerland). The standardized master cone (usually size 40) was lightly coated with the sealer and placed in the canal. Accessory Gutta-percha cones were added until the spreader penetrated into the coronal one-third of the root canal space. After obturation, excess Gutta-percha, 1 mm below the coronal surface was removed by a hot plugger. Of the 10 teeth used as controls, five positive controls were laterally condensed with Gutta-percha only (without sealer) and five negative controls were instrumented but not obturated.
and sealed externally with two layers of nail varnish, including the apex of the root and coronal access.

We evaluated the quality of root canal fillings by taking radiographs of all specimens in the buccolingual and mesiodistal directions. Specimens used in this experiment had radiographically well-compacted fillings that extended to 1 mm short of the apical foramen. Then all the specimens were stored in 100% humidity for 72 hours to allow full setting of the sealer. The external surfaces of the specimens in experimental and positive control groups were sealed with two coats of nail varnish (Christien dior, Paris, France), except for the apical 3 mm around the apical foramen and coronal surface of the root.

The obturated roots were inserted into an Eppendorf plastic tube (Eppendorf-Elkay, Shrewsbury, MA, Germany) with the apical part of the roots protruding through the end. Sticky wax was used in the junction between each tube and the coronal and middle portions of the roots to prevent leakage at the connection. Care was taken to ensure that no sticky wax covered the coronal end of the root.

The prepared Eppendorf tubes were placed in 30 ml glass tubes containing ten millimeters sterile phenol red broth with 3% lactose in a way that at least 2 mm of the root apex was submerged in the broth. The junction between the Eppendorf tube and glass tube was sealed tightly with cyanoacrylate glue (Razi, Iran) (Fig 1). The system was sterilized using 25 kGray gamma-ray. To ensure sterilization the whole system was incubated at 37°C for 3 days. Any test system that showed signs of turbidity in the lactose broth was discarded.

Finally, the specimens were further divided randomly into two subgroups inoculated by *S. sanguis* (ATCC10556) or the combination of *E. faecalis* (ATCC29212) and *C. albicans* (ATCC10231), resulting in four experimental groups of 25 teeth as follows: Group A, Group B, Group C, and Group D. In Group A, root canals obturated with AH 26 sealer and the coronal chambers inoculated with *S. sanguis*. In Group B, root canals obturated with AH 26 sealer and the coronal chambers inoculated with combination of *E. faecalis* and *C. albicans*. Root canals obturated with AH Plus sealer in Group C and the coronal chambers inoculated with *S. sanguis*. Root canals obturated with AH Plus sealer and the coronal chambers inoculated with combination of *E. faecalis* and *C. albicans* in Group D.

The microorganisms *S. sanguis*, *C. albicans*, and *E. faecalis* were cultivated on blood agar (Conda, Spain), Sabouraud Dextrose agar (Becton Dickinson, USA) and Brain Heart Infusion agar (BHI) (Mast, England), respectively. The microbial suspension was prepared by spots of microbial colonies in BHI broth. The coronal chambers of all apparatuses in each group were inoculated with 0.5 ml of microbial suspension containing 3×10^8 colony forming units (CFU)/ml of each microorganism using a sterile micropipette. The medium with microorganisms was changed every three days. The glass tubes were stored in an aerobic incubator at 37°C for 60 days. Throughout the experimental period, the color change of broth with phenol red from red to yellow, which indicated microbial penetration, was checked.

![Fig 2. Percentage of specimens with microbial penetration in the four experimental groups.](image-url)
daily. The days of microbial penetration were noted for evaluation. All specimens with evidence of turbid broth were removed from the experiment and a sample of them were cultured and incubated for 24 hours at 37°C to assure that it contained the same type of microbes inoculated in the coronal chambers. Microorganisms were identified by colony morphology, Gram staining and biochemical tests.

The data were statistically analyzed using Kaplan Myer and Log-rank tests. A value of P<0.05 was statistically significant.

RESULTS
In the test apparatus which was put in an incubator for three days before starting the experiment, there were signs of turbidity in five samples; four samples of the AH Plus group and one sample of the AH 26 group. These five samples were discarded.

Throughout the experiment, none of the specimens in the negative controls showed any changes in the lactose broth with phenol red, whereas all specimens in the positive controls caused a color change of phenol red to yellow.

Thirty-day leakage rate in four groups were as follows: Group A; 79.2 (SE=8.3%), Group B; 52.0 (SE=10%), Group C: 73.9 (SE=9.2%), Group D; 69.6 (SE=9.6%) (Fig 2).

The numbers of teeth showing microbial leakage until the end of the experimental period were seven out of 24 teeth in group A, 14 out of 25 in group B, seven out of 23 in group C and 13 out of 23 in group D (Table 1). There was no significant difference in the leakage rate among the four experimental groups (P=0.130).

DISCUSSION
This study was primarily aimed at the comparison of coronal leakage of two microbial markers in terms of leakage times and percentages. Furthermore, the sealing ability of AH 26 and AH Plus sealers against the same microbial markers was investigated. We used a combined E. faecalis and C. albicans suspension in an attempt to simulate in vivo conditions in which both microbes have been isolated from infected root canals associated with periapical lesions [14-16] and found in high quantities from failed root-filled teeth [18,19]. Peciuliene et al [15] showed that one-half of all root canals infected with C. albicans were associated with E. faecalis. S. sanguis is a facultative anaerobic bacterium frequently found in infected root canals [20-22].

Because of its small size, failure of this microorganism to leak through sealed root canals might indicate that larger microorganisms would not have this capability. S. sanguis was chosen in this study as a single bacterial strain to be compared with a combined bacterial-fungal suspension.

According to our results, specimens inoculated with a combination of E. faecalis and C. albicans (groups B and D) showed earlier microbial leakage compared to specimens inoculated with S. sanguis (groups A and C). In addition, the percentages of teeth showing microbial leakage at the end of the experimental period were greater in the former two groups.

The log-rank test did not show any significant difference in the leakage rate between the two microbial suspensions. E. faecalis was isolated from all specimens with yellow broth in groups B and D, while C. albicans was detected in few specimens. The larger size of C. albicans compared with E. faecalis could be an inhibitory factor for its penetration.

Although E. faecalis and S. sanguis are approximately the same size, the greater penetration of E. faecalis compared to that of S. sanguis might be related to its coexistence with C. albicans, which promotes the penetration of E. faecalis and prevents the penetration of C. albicans [23]. However, we could not distribute our findings to the E. faecalis - C. albicans coexistence because we used two different
bacteria in this study. In order to assess the effect of the concurrent presence of a fungus on the bacterial penetration, further investigations will be required using a single bacterial strain versus a combination of a fungus with the same bacteria.

In some studies, the specimens that filled with AH Plus have exhibited lower leakage values than specimens filled with AH 26 [9,24,25]. In contrast, other investigators have found higher leakage values for AH Plus sealer compared to AH 26 [5,26]. In current study, AH 26 and AH Plus sealers showed the same sealing ability after 60 days. Similar results have been reported previously [27-29].

We found a variability in the time it took the microorganisms to penetrate the entire root canal system. Microbial penetration through sealed root canals may depend on the balance between several influencing factors; increasing by sealer dissolution and setting shrinkage and decreasing by perhaps other yet unknown factors [4]. Variability in penetration time has also been reported in other studies [12,30]. The shape of the prepared canal, type of sealer and the nature of the solution used for coronal penetration have been stated as reasons for this variability [12].

CONCLUSION
Under the conditions of this in vitro study, there was no difference in the microbial penetration of AH 26 and AH Plus sealers up to 60 days. Also, there was no statistically significant difference in coronal leakage between Streptococcus sanguis per se with a mixture suspension of E. faecalis and C. albicans in root canals filled with Gutta-percha and either AH 26 or AH Plus sealers.

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