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Bacterial killing by several root filling materials and methods in an *ex vivo* infected root canal model

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Abstract

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Aim To evaluate the ability of two root canal sealers (Epoxy resin-based AH Plus® or polydimethylsiloxane-based GuttaFlow®) and five root filling techniques (continuous wave of condensation, Thermafil®, lateral condensation, matched taper single gutta-percha point, laterally condensed-matched taper gutta-percha point) to kill bacteria in experimentally infected dentinal tubules.

Methodology An infected dentine block model was used. One hundred and twenty extracted, single-rooted human teeth were randomly divided into 10 test (n = 10) and 2 control (n = 10) groups. The roots, except negative controls, were infected with *Enterococcus faecalis* for 21 days. The root canals were then filled using the test materials and methods. Positive controls were not filled. Sterile roots were used as negative controls. Dentine powder was obtained from all root canals using gates glidden drills using a standard method. The dentine powder was diluted and inoculated into bacterial growth media. Total colony-forming units (CFU) were calculated for each sample. Statistical analysis was performed using the Kruskal–Wallis and Mann–Whitney U test.

Results The epoxy resin-based sealer was effective in killing *E. faecalis* except when using Thermafil ($P < 0.05$), but the polydimethylsiloxane-based sealer was not effective in killing this microorganism except in the continuous wave group ($P < 0.05$).

Conclusions In the test model, AH Plus killed bacteria in infected dentine more effectively than GuttaFlow. The filling method was less important than the sealer material.

Keywords: AH Plus, antibacterial activity, filling methods, GuttaFlow.

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Introduction

Microorganisms in the root canal system play a primary role in the development of pulpal and periradicular infections (Kakehashi *et al.* 1965). The success or failure of root canal treatment depends on elimination of these microorganisms. Microorganisms in root canals should be eliminated through the physical removal of necrotic tissue and antimicrobial chemical therapy (Sundqvist & Figdor 1998). Chemo-mechanical procedures, cleaning, shaping and irrigation with disinfectants, may reduce the number of bacteria, but even after these procedures have been completed, some residual bacteria may remain in the root canal system (Byström & Sundqvist 1985, Ørstavik *et al.* 1991, Siqueira *et al.* 1997, Shuping *et al.* 2000).

Calcium hydroxide has been recommended as an intracanal medicament for total elimination of microorganisms (Byström *et al.* 1985). However, some studies show that calcium hydroxide may not ensure total elimination of microorganisms (Safavi *et al.* 1990, Ørstavik *et al.* 1991). In particular, bacteria

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resident in dentinal tubules, apical anatomy of root canal systems and isthmuses may not be completely eliminated by root canal treatment procedures (Siqueira 2001, Peters *et al.* 2002, Nair *et al.* 2005). Therefore, concern exists as to the fate and consequences of the remaining bacteria in the root canal system (Peters & Wesselink 2002). The remaining bacteria may be killed by the antibacterial activity of the sealer (Kaplan *et al.* 1999, Siqueira *et al.* 2000, Peters & Wesselink 2002) or they may be deprived of nutrition and space to multiply (Sundqvist & Figdor 1998).

Sundqvist & Figdor (1998) suggested that one of the goals of root canal filling is the entombment of remaining bacteria in the root canal system. Entombing of bacteria within the root canal system should result in all remaining bacteria being sealed by the root filling in dentinal tubules, lateral canals, apical ramifications and in the isthmuses so as to prevent their communication with the periodontium (Wu *et al.* 2006).

Today, numerous endodontic materials are available for filling the root canal system. Most techniques use a core material and sealer (Johnson & Gutmann 2006). Root canal sealers should provide a seal and have antibacterial activity (Grossman 1980). Indeed, it has been reported that various sealers have antibacterial activity (Saleh *et al.* 2004, Kayaoglu *et al.* 2005), and these sealers may help in the elimination of residual bacteria.

Various root canal filling methods have been developed to increase the success of root canal treatment. Studies evaluating the effect of filling techniques on residual microorganisms in root canal systems are limited. Although the fact that the remaining bacteria are not entombed in the root canal system with present root filling techniques and materials (Wu *et al.* 2006) has been accepted, some studies have shown that endodontic success could be achieved in infected root canal systems (Sjögren *et al.* 1997, Katebzadeh *et al.* 1999, 2000, Fabricius *et al.* 2006, Sabeti *et al.* 2006). However, it is unknown which method is more effective in the killing of remaining bacteria.

The aim of this study was to investigate the bacterial killing efficacy of two root canal sealers (the epoxy resin-based sealer AH Plus and polydimethylsiloxane-based sealer GuttaFlow) and five root canal filling techniques (continuous wave of condensation, Thermafil®, lateral condensation, matched taper single gutta-percha point, laterally condensed-matched taper gutta-percha point) against a mono-infection of *Enterococcus faecalis* in an experimental dentine tubule infection model.

Materials and methods

One hundred and twenty freshly extracted human maxillary incisor teeth were stored in 0.1% sodium hypochlorite for <6 months at 4 °C. Teeth with curved and immature roots were excluded. Calculus and tissue remnants were removed with curettes. Teeth were sectioned at or below the cemento-enamel junction with a diamond bur, and all of the roots were adjusted to 13 ± 0.5 mm.

A size 15 K-file (Mani Inc, Tochigi, Japan) was placed into the canal until its tip was observed at the apical foramen. The working length was established 1 mm short of the root length. Each root canal was instrumented with the ProTaper rotary system (Dentsply Maillefer, Ballaigues, Switzerland) to an apical size of F5 (50) using the crown-down technique. During instrumentation, 2 mL of 1% sodium hypochlorite solution (Caglayan Chemistry, Konya, Turkey) was used for irrigation between each file. Finally, all root canals were irrigated with 5.25% sodium hypochlorite, 17% EDTA solution and distilled water for 3 min each in an ultrasonic bath (Bandelin Electronic, Berlin, Germany) to remove the smear layer. All of the roots were sterilized through autoclaving for 20 min at 121 °C and prepared for infection as described by Saleh *et al.* (2004).

The streptomycin-resistant *E. faecalis* (A197A) strain (isolated in Finland by Sirén *et al.* 1997) was used as the test microorganism. The specimens were randomly distributed into 12 groups, and each group was placed into 10 mL TSB (Biomerieux, Marcy I'Etoile, France) with 2 mg mL^{-1} streptomycin inoculated with 25 μL of a 24-h-old *E. faecalis* suspension, except one group. Bacterial numbers were standardized spectrophotometrically (Biotec Instruments Inc, Winovski, VT, USA) to OD_{600} = 0.6 before the specimens were placed into bacterial suspension. The bacterial suspension was changed every 2 days for a period of 3 weeks. The purity of the cultures was regularly checked by Gram staining. Bacterial penetration into the dentinal tubules was confirmed using scanning electron microscope (Fig. 1).

After the infection period, all root canals were dried with paper points and the 11 infected groups were assigned to 10 experimental groups, one positive control group and one sterile group served as the negative control (n = 10 per group). The randomiza-

tion method consisted of allocation by one operator who was blinded to the experimental materials and methods.

Groups 1 and 6: AH Plus or GuttaFlow and continuous wave of condensation

Root canals were filled using the BeeFill 2in1 System (VDW; Aseptico, Woodinville, WA, USA) according to the manufacturer's instructions. For the continuous wave of condensation technique, ProTaper F5 gutta-percha was selected and adapted to working length. This gutta-percha point was coated with the epoxy resin-based sealer AH Plus (Dentsply De Trey, Konstanz, Germany) or the polydimethylsiloxane-based sealer GuttaFlow (Coltene/Whaledent, Altstätten, Switzerland) prepared according to manufacturer's instructions and inserted into the root canal. Then, the BeeFill 2 in 1 system plugger was heated to 200 °C and placed through the filling with gentle, apical compression to a depth 5 mm short of the working length for 4 s. The heated plugger was allowed to cool for 10 s and then removed after applying 1 s of heat. After this, vertical condensation was applied using an appropriate manual plugger. The empty part of the root canal was backfilled using the BackFill component of the BeeFill 2in1 system with 23-gauge needle tips at 170 °C, and the root filling was completed to the level of the canal orifice.

Groups 2 and 7: AH Plus or GuttaFlow and Thermafil (n = 10)

A number F5 ProTaper Thermafil Obturator was heated in the ThermaPrep Oven (Dentsply) for the recommended time. The epoxy resin-based sealer or the polydimethylsiloxane-based sealer was placed at the coronal orifice. Then, the pre-heated ProTaper Thermafil was inserted slowly to the working length. The excess gutta-percha was condensed vertically. After the gutta-percha led cooled (4 min), a sterile blade was used to cut the plastic carrier 1 mm above the canal orifice and excess gutta-percha was removed.

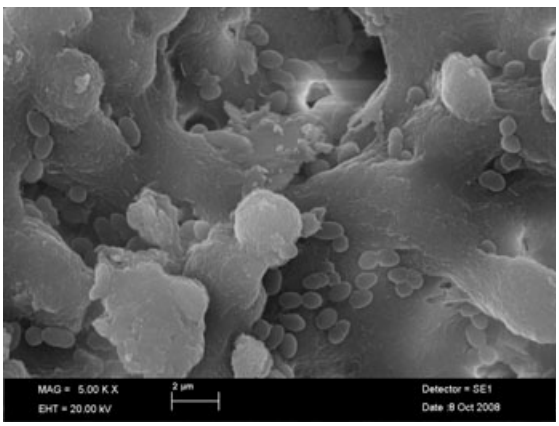

Figure 1 Infection of dentinal tubules after 3 weeks.

Groups 3 and 8: AH Plus or GuttaFlow and lateral condensation (n = 10)

A size 50 master gutta-percha point (Diadent, Seoul, Korea) was fitted at the working length. The sealer was placed into the root canal using lentulo spiral. The master point was lightly coated with the epoxy resin-based sealer or the polydimethylsiloxane-based sealer and placed in the root canal and condensed by the spreader to the full working length. Auxiliary sizes 25 and 20 cones were condensed until it was not possible to place another accessory cone further than 3 mm into the root canal. The excess gutta-percha was removed with a heated instrument at the canal orifice, and vertical condensation was applied with a cold plugger for 5 s.

Groups 4 and 9: AH Plus or GuttaFlow and matched taper single gutta-percha point (n = 10)

A number F5 ProTaper gutta-percha point (Dentsply Maillefer, Ballaigues, Switzerland) was fitted to the working length. The epoxy resin-based sealer or the polydimethylsiloxane-based sealer was placed into the root canal using a lentulo-spiral instrument. The gutta-percha point was lightly coated with the sealer. The excess gutta-percha was removed with a heated instrument at the canal orifice, and vertical condensation was applied with a cold plugger for 5 s.

Groups 5 and 10: AH Plus or GuttaFlow and laterally condensed-matched taper gutta-percha point (n = 10)

After a number F5 ProTaper gutta-percha point was fitted to the working length as in group 4, lateral condensation was performed using sizes 25 and 20 accessory gutta-percha points until the spreader could not be introduced more than 3 mm into the root canal. The excess gutta-percha was removed with a heated instrument at the canal orifice, and vertical condensation was applied with a cold plugger for 5 s.

Positive control

The 10 infected root canals were dried with paper points and not filled.

Negative control

Ten sterile root canals were used to show reliability of test procedures.

All samples were placed into sterile microplates and stored at 37 °C and 100% humidity for 1 week to allow the sealers to set. Then, a 3 mm apical portion of the samples was resected, and dentine blocks were acquired. Root canal fillings were removed using a size 2 gates glidden bur, and root canals were prepared using sizes 3, 4 and 5 gates glidden burs. Dentine powder was obtained from each sample and collected on sterile aluminium foil. For each sample, new sterile gates glidden burs were used.

The dentine powder was placed into a glass bottle containing 2 mL phosphate buffered saline (PBS; Sigma-Aldrich, Steinhelm, Germany) and shaken for 10 s. This mixture was diluted to 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} concentrations, and each concentration was shaken for 10 s. Two drops of suspension (25 μL) from each concentration were placed onto Tryptic Soy Agar (TSA; Biomerieux, Marcy I'Etoile, France) plates with 2 mg mL^{-1} streptomycin. The plates were incubated for 48 h at 37 °C. Colony-forming units (CFU) were calculated and converted to their $\log_{10}$ values.

A kurtosis evaluation of data was performed; the kurtosis values ranged from −1.29 to 3.44, showing that the data were not normally distributed. The data were, therefore, analysed using the Kruskal–Wallis test with a significance level of $P < 0.05$. Statistically significant differences were found among the groups (sealers and root filling techniques). Then, the Mann–Whitney U test was used for *post hoc* analysis ($P < 0.05$).

Results

The mean and median $\log_{10}$ CFU values, standard deviations and ranges are shown in Table 1 and Fig. 2. The median $\log_{10}$ CFU value was 4.24 for the positive control, and bacteria were recovered from all samples. The median $\log_{10}$ CFU value was 0 for the negative controls, indicating freedom from environmental contamination during the procedure.

The Kruskal–Wallis test showed significant differences among the AH Plus groups ($P < 0.001$). Multiple comparisons with Mann–Whitney U test indicated that the median $\log_{10}$ CFU value for the Thermafil® group was significantly higher than that of the other AH Plus groups and of the negative control ($P < 0.05$). The epoxy resin-based sealer killed all bacteria in dentinal tubules (median CFU = 0), except in the Thermafil® group (median CFU = 2.99). The bacteria were completely killed in just two samples of this group. Also, as a result of multiple comparisons, the Thermafil® group was found to be significantly different from the positive control ($P < 0.05$). Additionally, other AH Plus® groups were not significantly different from the negative control ($P > 0.05$).

Bacteria were isolated from all samples filled with the polydimethylsiloxane-based sealer regardless of the filling techniques. The Kruskal–Wallis test indicated that there were significant differences among the

Table 1 Mean and median $\log_{10}$ colony-forming unit values, standard deviations (SD) and ranges

Groups	*n*	Means	SD	Medians	Range
1. AH Plus + Continuous wave of condensation	10[a]	0.00	0.000	0.00	0.00–0.00
2. AH Plus + Thermafil	10[b]	2.64	1.476	2.99	0.00–4.06
3. AH Plus + Lateral condensation	10[a]	0.00	0.000	0.00	0.00–0.00
4. AH Plus + Matched taper single gutta-percha point	10[a]	0.00	0.000	0.00	0.00–0.00
5. AH Plus + Laterally condensed-matched taper gutta-percha point	10[a]	0.00	0.000	0.00	0.00–0.00
6. GuttaFlow + Continuous wave of condensation	10[c]	2.99	0.349	2.90	2.60–3.56
7. GuttaFlow + Thermafil	10[c]	3.95	0.413	4.07	3.08–4.43
8. GuttaFlow + Lateral condensation	10[c]	3.90	0.379	3.93	3.32–4.37
9. GuttaFlow + Matched taper single gutta-percha point	10[c]	4.04	0.249	4.04	3.64–4.37
10. GuttaFlow + Laterally condensed-matched taper gutta-percha point	10[c]	4.00	0.384	4.10	3.08–4.43
11. Positive control	10[c]	4.32	0.389	4.24	3.78–4.92
12. Negative control	10[a]	0.00	0.000	0.00	0.00–0.00

[a]All specimens were negative.
[b]Two specimens were positive, and eight specimens were negative.
[c]All specimens were positive.

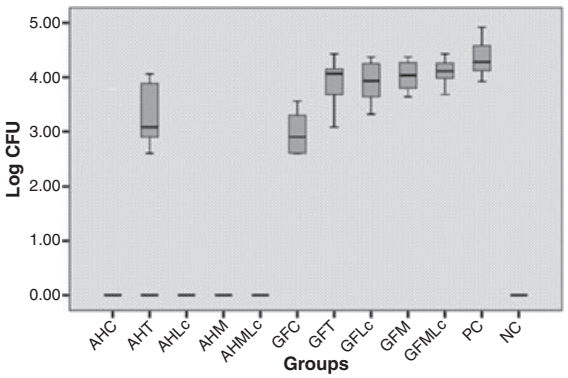

Figure 2 Antibacterial activity of groups against *Enterococcus faecalis*. (AHC, AH Plus + Continuous wave of condensation; AHT, AH Plus + Thermafil; AHLc, AH Plus + Lateral condensation; AHM, AH Plus + Matched taper single gutta-percha point; AHMLc, AH Plus + Laterally condensed-matched taper gutta-percha point; GFC, GuttaFlow + Continuous wave of condensation; GFT, GuttaFlow + Thermafil; GFLc, GuttaFlow + Lateral condensation; GFM, GuttaFlow + Matched taper single gutta-percha point; GFMLc, GuttaFlow + Laterally condensed-matched taper gutta-percha point; PC, positive control; NC, negative control).

GuttaFlow groups ($P < 0.001$). According to the Mann–Whitney *U* test, the single group found to be significantly different from the other GuttaFlow groups was that of continuous wave of condensation ($P < 0.05$). The median $\log_{10}$ CFU value for GuttaFlow *and* continuous wave of condensation was 2.90, which was significantly lower than that of the other GuttaFlow groups and positive controls. The other GuttaFlow groups were not significantly different from each other or from the positive control ($P > 0.05$).

Discussion

In this study, the dentine block model developed by Haapasalo & Ørstavik (1987) was used with some modifications. This method enables assessment of antibacterial activity in dentinal tubules and accessory canals at different levels. This model is used especially for evaluation of antimicrobial effects of root canal sealers and medicaments. In this study, the apical 3-mm sections of roots were removed to eliminate differences arising from the apical delta and apical lateral canals (Vertucci 1984). Also, this process may be necessary to prevent contamination with external surfaces of the root because the diameter of the apical end of root was smaller than the diameter of the gates glidden burs used to obtain the dentine powder.

Enterococcus faecalis (A197A), a root canal isolate from a persistent endodontic infection (Sirén *et al.* 1997), was chosen as the test microorganism. *E. faecalis*, a facultative microorganism, has been reported as the most commonly identified species in root canals of failed root filled teeth (Love 2001, Pinheiro *et al.* 2003). In the root canal microbiota, it is one of the most resistant microorganisms (Siqueira & de Uzeda 1996). Siqueira *et al.* (1996) showed that it can penetrate dentinal tubules. Because of these properties in numerous previous studies that investigated the disinfection of dentinal tubules, *E. faecalis* has been used as the test microorganism (Haapasalo & Ørstavik 1987, Safavi *et al.* 1990, Saleh *et al.* 2004).

Haapasalo & Ørstavik (1987) found that 3 weeks of incubation with *E. faecalis* produced a dense infection up to 300–400 μm within the dentinal tubules. Long-term infection mainly ensures more tubules become infected, whereas the average depth reached by bacteria in the dentinal tubules has been shown to increase slowly with time (Haapasalo & Ørstavik 1987). In a clinical study, Peters *et al.* (2002) found that the median total CFU counts were 7.6×10^4 prior to treatment of root canals of teeth with periapical lesions. Similarly, the median $\log_{10}$ CFU value for positive control samples was 4.24 ($\sim 1.7 \times 10^4$) in the present study, albeit in terms of bacterial load rather than diversity, demonstrating some similarity to the clinical infection. Sjögren *et al.* (1991) and Ørstavik *et al.* (1991) also found CFU counts of 9.8×10^4 and 4×10^5 before the start of treatment.

In the current study, AH Plus and GuttaFlow sealers showed a different antibacterial effect on *E. faecalis*. In all AH Plus groups except one (AH Plus *and* Thermafil®), viable residual microbial cells were not detected. The polydimethylsiloxane-based sealer was found ineffective against *E. faecalis* except in one group (GuttaFlow and continuous wave of condensation). The results of the present study are compatible with several studies. Cobankara *et al.* (2004) reported that AH Plus was inhibited *E. faecalis*, but RoekoSeal, the precursor of GuttaFlow, had no antibacterial effect against this microorganism. In another dentine block model study, Saleh *et al.* (2004) showed that AH Plus in root fillings killed all bacteria in the dentinal tubules within a 300 μm zone around the root canal, but RoekoSeal had limited antibacterial effect against *E. faecalis*.

Mickel *et al.* (2003) reported that AH Plus had no antibacterial effect on *E. faecalis*, in an agar diffusion test. However, they stated that the blood agar plates were not similar to the environment in the dentinal

tubule. Dentine block models may be more relevant clinically (Kayaoglu *et al.* 2005).

The antibacterial effect of resin-based sealers may be associated with bisphenol A diglycidyl ether, which was previously identified as a mutagenic component of the resin-based material (Heil *et al.* 1996). The component caused the AH Plus sealer to have antibacterial qualities.

Limited study has been conducted to investigate antibacterial activity of the GuttaFlow root canal sealer. The present data showed that the polydimethylsiloxane-based sealer, except in one group, had no antibacterial effect on *E. faecalis* and was not different from the positive control. The results were consistent with a study concerning the antibacterial activity of GuttaFlow. Mohammadi & Yazdizadeh (2007) reported that AH-26 was more effective than RoekoSeal and GuttaFlow in reducing *Staphylococcus aureus* and *Streptococcus mutans.*

Most root canal sealers have antibacterial components. Root canal sealers with strong antibacterial activity have been found to be cytotoxic and even mutagenic (Geurtsen & Leyhausen 1997). Previously, the cytotoxicity of AH Plus has been reported, but the results were controversial. Even though Camps & About (2003) reported that AH Plus did not have cytotoxic properties, Cohen *et al.* (2000) and Miletić *et al.* (2005) claimed it had a strong toxic effect. Bouillaguet *et al.* (2006) reported that GuttaFlow was significantly less toxic than AH Plus.

In this study, when the epoxy resin-based sealer was used as a sealer, only the Thermafil group was found to be significantly different from the other experimental root canal filling methods. Four root canal filling methods, using the epoxy resin-based sealer, killed all bacteria in the dentinal tubules. On the other hand, these results were obtained in only two samples of Thermafil groups, and bacteria were isolated from 80% of the root canals. This may be due to differences in the placement of the sealer. While the root canal sealer was placed into root canals using a lentulo-spiral instrument in the lateral condensation, matched taper single gutta-percha point and laterally condensed-matched taper gutta-percha point groups, the sealer coated gutta-percha point was inserted into root canal in the continuous wave of condensation group, in accordance with the manufacturer's instructions. However, the root canal sealer was placed at the coronal orifice in the Thermafil group. It has been reported that sealer extrusion through the apex is a common condition with the Thermafil method, and it is one of the major drawbacks of this method (Lares & elDeeb 1990, Dummer *et al.* 1994). Thus, it was recommended that the sealer should be applied only to the canal orifice (Carrotte 2004). Other authors have also applied the sealer only to the canal orifice (Yucel & Ciftci 2006), but this procedure results in very small volumes of sealer on the root surface; in the middle third of the root canal, especially, there is almost no sealer found (Guigand *et al.* 2005). This may explain why the Thermafil technique was ineffective for the elimination of residual bacteria in dentinal tubules compared with the other root canal filling methods tested.

In the GuttaFlow groups, the continuous wave of condensation group was significantly different from the other filling methods. Bacteria were isolated from all samples, but the median number of viable bacteria was lower than that of all other filling methods and of the positive control. This may be due to the efficacy of the continuous wave of condensation technique as a function of its potential to reduce the space and nutrition for the multiplication of remaining microorganisms (Sabeti *et al.* 2006). Gencoglu *et al.* (1993) showed that the continuous wave of condensation was more effective than other methods in the filling of lateral canals and dentinal tubules.

The results of the present study revealed that the root filling methods with the polydimethylsiloxane-based sealer, except in the continuous wave of condensation group, did not affect the total number of viable bacteria, which were not different from the positive control group. This may be due to the poor antibacterial properties of the polydimethylsiloxane-based sealer.

Sjögren *et al.* (1997) reported a 68% success rate for teeth with a positive culture at the time of root canal filling. Peters & Wesselink (2002) claimed that the presence of positive culture at the time of root filling did not affect the outcome of endodontic therapy. The data from the present study demonstrated that residual bacteria may be rendered uncultivable within the test zone around the root filled canal, especially if a sealer with strong antibacterial activity, such as AH Plus, is used.

Conclusion

The present study confirmed that the AH Plus root canal sealer was effective in rendering the test *E. faecalis* cells in this *ex vivo* dentine infection model uncultivable or dead, whilst GuttaFlow was ineffective in achieving this. The Thermafil method as applied was not effective in controlling the infecting bacteria.

Selection of a root canal sealer may be more important than the root canal filling method for elimination of bacteria.

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