Original Article

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Bacterial leakage in root canals filled with calcium silicate sealer-based technique and post spaces prepared

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Objective: To compare bacterial leakage in root canals filled with sealer-based technique in three immediate or delayed post space preparation methods.
Materials and Methods: Seventy-five palatal root canals were prepared and filled with calcium silicate or bioceramic (BC) impregnated gutta-percha cone and BC sealer using the sealer-based technique. The specimens were divided into three groups according to post space preparation methods: (1) I-HE: immediate preparation by heat, (2) I-SC: immediate preparation by sectional cone obturation, and (3) D-DR: delayed preparation by drilling. The specimens with post spaces and 4-mm root filling material remaining in the apex were tested for bacterial leakage of Enterococcus faecalis using two-chamber model for 45 days. The chi-square test and log-rank test were employed to analyze and compare the difference among the three groups
Results: Within 45 days, bacterial leakage occurred in 40-44% of the root canals, and the occurrence rates among the three post space preparations were not significantly different (P>0.05).
Conclusion: Three post space preparation techniques, immediate with heat, sectional-cone filling, or delayed drilling, did not show significant difference in bacterial leakage in the root canals filled with calcium silicate sealer-based technique.

Keywords: bacterial leakage, bioceramic, calcium silicate, post space preparation, root canal sealer

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Introduction

The majority of root canal-treated teeth have extensive loss of tooth structures and require restoration with the use of a post and core. When a post space is prepared, at least 4 mm of gutta-percha root canal filling must remain to maintain an adequate apical seal [1]. A post space can be immediately prepared by down packing the root canal filling with a heated plugger [2-4] or delayed prepared by drilling with a Peeso reamer [5,6]. However, a high number of post space-prepared root canals filled with gutta-percha and epoxy resin or zinc oxide eugenol-based sealer showed bacterial leakage within 45 days [7, 8].

To increase resistance to bacterial leakage, the root canal filling material and technique should be improved. Recently, a new bioceramic (BC) or calcium silicate-based root canal sealer (TotalFill BC Sealer, FKG Dentaire SA, La Chaux de Fonds, Switzerland) has been introduced. The root canal sealer is used with a calcium-silicate impregnated/coated cone, which might bond together [9]. The sealer might be able to bond with dentin by

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induction of hydroxyapatite formation and cause a chemical interaction [10]. According to the manufacturer's instructions, the sealer-based root canal obturation technique recommends using a matched-taper single cone and thick sealer. This technique eases the procedure and reduces the force during obturation. Use of this BC sealer-based technique provides resistance to bacterial leakage similar or superior to that achieved from the conventional technique using an epoxy resin-based sealer [11].

Apical sealing for root canals obturated with this BC sealer-based technique followed by post space preparation has not previously been reported. Moreover, the proper method or time for post space preparation to maintain the sealing ability is still controversial. Immediate preparation by down-packing/removal of the root canal filling using heat or delayed preparation using a drill is routinely performed. However, using heat with the sealer-based technique may negatively affect the BC sealer by reducing the flow ability and adaptation with root canal walls [12]. In contrast, delayed post space preparation by drilling with a bur might induce cracks in a set BC sealer and disrupt the bond between the sealer and dentin/cone.

In an attempt to solve these problems, the sectional cone technique using a 4-5 mm sectioned cone for apical root canal obturation has been proposed. In this technique, the sectional cone is carried and seated into the sealer-coated root canal by an endodontic plugger, which simultaneously obturates the root canal and creates a post space without using heat or drilling. Thus, post space preparation using the sectional cone technique may enhance the sealing ability of obturation.

The objective of this study was to compare bacterial leakage in the root canals, filled using the BC sealer-based technique, among three different methods of post space preparations, i.e., immediate preparation with heat, immediate preparation by the sectional cone technique, and delayed preparation by drilling.

Materials and Methods

The study protocol was approved by the Committee of the Faculty of Dentistry and Faculty of Pharmacy, Mahidol University Institutional Review Board (MU-DT/PY-IRB 2017/047.0311).

Seventy-nine caries-free, extracted human maxillary molars, with straight palatal roots and mature apices were collected and stored in 0.1% thymol solution. The teeth were immersed in 2.5% sodium hypochlorite (NaOCl) for 5 min to disinfect and dissolve soft-tissue remnants. The palatal roots were inspected using a dental operating microscope (OPMI PICO, ZEISS, Göttingen, Germany) under 24x magnification, and the roots with crack lines were excluded.

A length of 10 mm from the root apex was measured, and the crown was removed at that level with a high-speed bur under water spray. The working length was determined by passing a no. 10 K-file (Kerr, Romulus, MI, USA) at the apical foramen, and 0.5 mm was subtracted from the file length. To control the apical diameter, the roots with apical foramen larger than a no. 25 K-file were excluded. A no. 15 K-file was used to confirm the glide path of the root canal. Root canal preparation was performed using the RaCe Rotary NiTi files (FKG, Le Chaux-of-Fonds, Suisse, Switzerland), and 17% EDTA paste (RC prep, Premier Dental Products, King of Prussia, PA, USA) was used as a lubricant. The apical size was prepared using the crown-down technique to a master apical file size 40/.06. Irrigation was performed using 2.5% NaOCl solution for a total volume of 15 ml. To remove the smear layer, the root canals were finally irrigated with 3 ml of 17% EDTA solution (M Dent, Bangkok, Thailand) for 1 min and 5 ml of 2.5% NaOCl. The canals were dried with three paper points.

The prepared root canals were randomized into three experimental groups for post space preparation with twenty-five roots each and two control groups with two roots each. In the

experimental groups, the root canals were filled by the sealer-based technique using a single matched-taper master cone size 40/.06 TotalFill BC gutta-percha and BC sealer; post spaces were prepared by the three different techniques as follows.

Group 1, I-HE: immediate preparation with heat

The sealer was injected through the intracanal tip into the canal. The tip was slowly withdrawn while injecting the sealer into the canal orifice. To optimize sealer distribution, a Lentulo spiral no. 35 (Dentsply Maillefer, Ballaigues, Switzerland) was used in a 16:1 gear reduction handpiece with a torque-controlled electric motor (NSK-Nakanishi, Kanuma, Tochigi, Japan) using 700 rpm rotating speed to load the sealer into the canals up to 1 mm short of the working length. The master cone was coated with the BC sealer and slowly inserted into the canal to the working length. The quality of root canal filling was checked radiographically. If any voids were present or the filling was short of the working length, the specimen was excluded and replaced with a newly prepared specimen. To create a post space, the master cone was down-packed using the continuous wave technique with a System B heat source, using a .06 taper heat carrier (SybronEndo, Orange, CA, USA), to the level of 4 mm from the working length. Remnants of the sealer on the canal walls were cleaned using dry paper points.

Group 2, I-SC: immediate preparation with the sectional cone technique

The BC sealer was injected from the tip and loaded into the root canal using the Lentulo spiral. The BC cone was tested and cut to a 4-mm length before it was inserted into the canal and seated to the working length using an endodontic plugger no. 1/2 (Dentsply, Tulsa, OK, USA). Remnants of sealer were cleaned as previously described.

Group 3, D-DR: delayed preparation with drills

Root canal filling was performed as in group 1, but the master cone was seared off at the canal orifice. The filled roots were stored as described below for 7 days. Afterward, the post space was prepared with a size no. 2 Peeso reamer (Mani, Tochigi, Japan), leaving 4 mm of obturation at the apical third, and it was further drilled with a size 0.5 post drill (DT Light-Post drill, Dentaires, St. Egreve, France) to create a proper size of post space.

Negative control: The root canal was filled with the sealer-based technique using the BC cone size 40/.06, but the entire root and apical foramen were coated with two layers of nail varnish.

Positive control: The root canal was filled with the BC cone size 40/.06 without sealer.

The roots in all groups were stored in an incubator at 37°C with 100% humidity for 7 days to allow the sealer to completely set. In groups 1 and 2, the prepared post spaces were further shaped using a size 0.5 post drill (that used in group 3) to create and control a similar size for the post space. The post space was rinsed with distilled water and dried with absorbent paper points. Radiographs in bucco-lingual and mesio-distal views were obtained to evaluate the quality of the apical root canal filling and any remnants of the sealer on the root canal walls.

Bacterial leakage test

The bacterial leakage test was based on the method used by Torabinejad et al. [13] The filled root was put in a sterile upper chamber of a 1.5-ml microcentrifuge tube (Treff Lab, Schweiz, Switzerland) that was assembled with a sterile lower chamber of a 10-ml glass vial. Two layers of nail varnish (Revlon, New York, NY, USA) were applied to cover the root surface except for the 3-mm area around the apical foramen. Brain heart infusion (BHI) broth (Difco Laboratories, Detroit,

MI, USA) was poured into the glass vial with 2 mm of the root apex submerged in the BHI broth (Fig. 1) and then sterilized using an autoclave at 121°C and 15 psi for 20 min. To confirm sterile conditions, the specimens were incubated in anaerobic conditions at 37°C for 24 h.

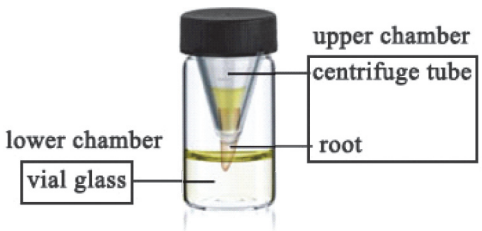

Figure 1 The two-chamber bacterial leakage model.

Enterococcus faecalis (ATCC® 29212™) was rehydrated with 0.5 ml of BHI broth and subcultured in BHI agar under incubation at 37°C for 24 h. The bacterial clusters were transferred into 5 ml of BHI broth and incubated at 37°C for an additional 24 h. The culture in BHI broth was diluted with fresh medium to obtain the turbidity of a 0.5 McFarland standard ($1x10^8$ cells per ml). The model was put into a 5% CO_2 incubator at 37°C and 100% humidity. To ensure the viability of *E. faecalis* throughout the experimental period, 200 of the medium was changed using an aseptic method every 3 days in the upper chamber. By examining turbidity in the lower chamber, incidences of bacterial leakage were assessed daily for 45 days. The leaked bacteria were investigated by gram staining and colony morphology on trypticase soy agar (TSA) to confirm the presence of *E. faecalis*.

From the three experimental groups, the frequency (%) of leaked samples was described, and the time of leakage was noted in days. The chi-square test was employed to analyze and compare the number of leaked samples among the three groups ($P < 0.05$). To evaluate the time to bacterial leakage, a Kaplan-Meier survival plot was used to visualize the survival rate from bacterial leakage, and the log-rank test was utilized to identify any significant difference, with a significance level set at 0.05.

Results

None of the negative control samples showed leakage, and all of the positive control samples demonstrated turbidity, indicating leakage within the first 24 hours. The broth with bacterial leakage were subcultured in brain heart infusion agar and gram staining was used to confirm that no contamination by other bacteria. All broth that presented turbidity showed the presence of E. faecalis.

Overall, bacterial leakage occurred in 40-44% of the samples, with the leakage time from 2 to 45 days. The number of leaked samples and the leakage times are summarized in Table 1. I-HE, I-SC and D-DR presented leakage in 10, 11 and 10 of 25 samples, respectively. There was no significant difference in the number of leaked samples among the three experimental groups ($P > 0.05$). The Kaplan–Meier survival curves are shown in Fig. 2. There were no significant differences in the survival rate from leakage among the three experimental groups at any time points ($P > 0.05$).

Table 1 Frequency (%) of leaked specimens and the leakage periods.

Group	% Leakage (number of leaked samples/total)	Leakage period (days)
1. I-HE: immediate with heat	40 (10/25)	5-41
2. I-SC:immediate with sectioned cone	44 (11/25)	2-44
3. D-DR: delayed with drills	40 (10/25)	5-45

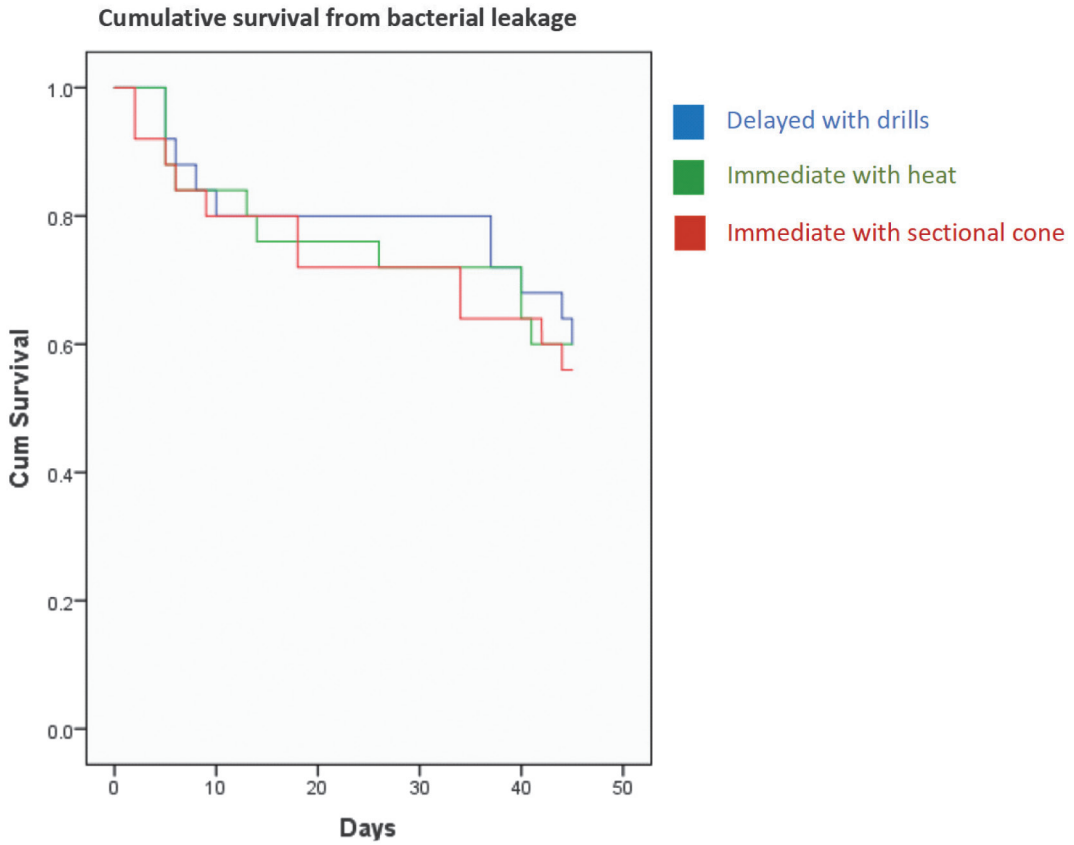

Figure 2 Kaplan-Meier survival curves of bacterial leakage in the obturated root canals for the three post space preparation methods.

Discussion

In our study, approximately half of the specimens showed bacterial leakage within 45 days in the root canals filled with the calcium silicate-based sealer and with post space preparation. In previous studies, the root canals obturated using the calcium silicate-based sealer, but those without postspace preparation showed bacterial leakage in only 25% of samples within 60 days [11]. The shorter length of remaining root canal fillings after post space preparation might have caused the higher proportion of bacterial leakage in our study.

No previous study has reported the effect of the post space preparation technique on the leakage for this BC sealer. The three techniques used in this study for root canals filled with the BC sealer-based technique showed similar bacterial leakage among the immediate (with heat and without heat) and delayed (with drilling) post space preparations.

For the immediate preparation, similar bacterial leakage was found when the post space was prepared with or without heat in this study. Without using heat in the sectional cone technique, the flow of the sealer was not negatively affected by the increased temperature [12]. However, the 4-mm length of the sectioned BC cone may cause a lower hydraulic force during root canal obturation, for which less sealer penetration/adaptation might be expected when compared to obturation with the full-length of the BC cone. When using heat to prepare a post space, the increased temperature might reduce the flow ability of the BC sealer and decrease sealer adaptation to root canal walls [12]. Even the down-packing of the melted BC cone during warm vertical compaction creates a force

to push the sealer [14]. This technique did not improve the resistance to leakage in our study.

In our study, the immediate and delayed post space preparation with drilling showed similar bacterial leakage. The sealing ability of the root canals with the immediate preparation by warm vertical compaction or the sectional cone technique did not improve as described above. For the delayed preparation, root canal obturation with a full-length BC cone might cause more hydraulic pressure to enhance sealer penetration/adaptation. Moreover, no heat was used, so the flowing capacity of the sealer was not affected. Nevertheless, the delayed post space preparation by drilling after the sealer was completely set might disrupt the bond between the sealer and dentinal wall [2]. This phenomenon might explain why the delayed preparation did not provide better leakage resistance than the immediate preparation in our study.

Half of the bacterial leakage was found within the first 10 days of the experiment. However, the trend in the bacterial leakage rate still gradually occurred throughout the experiment. Therefore, root canals filled with the BC sealer-based technique and with post space preparation must be protected against bacterial contamination by preparation under rubber dam isolation and placement of coronal restoration.

In this study, bacterial leakage was found to not different among the three groups, but preparing the post space by the immediate sectional cone method (I-SC) required more effort. For this technique, the insertion of a 4-mm BC cone to the working length was difficult to control. In addition, complete removal of the unset sealer in the coronal part was also a problem. If the immediate preparation is planned, using heat to down-pack the root filling material as a part of the obturation procedure and simultaneously creating the post space seems to be more practical. This technique, without any drilling, also provides an additional benefit in preventing root perforation and contamination due to rubber dam isolation. On the other hand, the delayed preparation by drilling was clinically faster, but overpreparation or root perforation is possible if it is prepared without caution. From a clinical point of view, post space preparation in a root canal filled with the BC sealer-based technique is able to be performed either immediately using heat for down-packing by the endodontist or delayed using a drill by the prosthodontist because the bacterial leakage is not different between these techniques.

Numerous methods are used for testing the sealing ability of filling materials, but none of them can be considered to be a gold standard [15]. Dye penetration or fluid infiltration tests are not clinically relevant. The bacterial leakage test seems to be more clinically applicable. However, the result of an *in vitro* bacterial leakage test cannot be directly extrapolated to clinical situations because the leakage of few bacteria may cause positive results in the laboratory test while human immunity potentially eliminates the low number of leaking bacteria *in vivo*. Furthermore, the minimum level of bacteria that causes endodontic failure has not yet been identified.

In conclusion, the post space preparation techniques, either immediate with heat or without heat (sectional cone) or delayed by drilling, were not significantly different in bacterial leakage in the root canals filled using the bioceramic sealer-based technique.

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Conflict of Interest and Source of Funding Statement

The authors deny any conflicts of interest related to this study.

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