

Effect of immediate and delayed post space preparation on coronal bacterial microleakage in teeth obturated with a methacrylate-based sealer with and without accelerator

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ABSTRACT: Purpose: To investigate the sealing properties of root fillings with resin-coated gutta-percha cones and a methacrylate-based resin endodontic sealer with and without an accelerator component in root canals subjected to immediate or delayed post space preparation. **Methods:** Forty-eight extracted human teeth with single straight root canals were treated endodontically. Specimens were then assigned to four groups of 10 teeth each (n= 10). After autoclaving, the following operative procedures were carried out under strict aseptic conditions. In Group 1 the root canals were filled with resin-coated gutta-percha cones and a methacrylate based resin endodontic sealer (EndoREZ). Post space preparations were performed 2 minutes after the sealer had set. In Group 2 the root canals were filled as in Group 1 but with the addition of a chemical accelerator. The post space preparations were also performed 2 minutes after the sealer had set. Groups 3 and 4 were filled as in Groups 1 and 2 respectively, however the post space preparations were done 7 days after the root canal filling was completed. One positive and one negative control tooth per group was added. All specimens were subjected to a coronal bacterial leakage of *E. faecalis* during a 60-day period using a dual chamber microbial leakage model. Data was analyzed among groups with the Kaplan-Meier survival analysis while significant pairwise differences were analyzed with the log-rank test ($P < 0.05$). **Results:** No significant differences ($P > 0.05$) in bacterial leakage were observed between Groups 1 and 2 and between Groups 3 and 4. However, Groups 1 and 2 differed significantly from Groups 3 and 4 ($P < 0.05$). (*Am J Dent* 2010;23:116-120).

CLINICAL SIGNIFICANCE: The findings demonstrated that regardless of whether an accelerator was used or not, a delayed post space preparation resulted in more pronounced and faster coronal bacterial leakage compared to immediate post preparation after the sealer had set. Since the accelerator *per se* did not affect the leakage pattern of the sealer, its use can be recommended to shorten the setting time, thus allowing the practitioner to prepare the post space during the same appointment as the endodontic treatment.

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Introduction

After completion of root canal treatment, a post and core is frequently required in order to finalize the permanent prosthetic restoration. This procedure calls for partial removal of the root canal filling material. During post space preparation, it is important not to disturb the apical seal provided by the remaining filling material. According to De Cleen,¹ a post space preparation should preferably be performed after the root canal has been filled during the same session and while the tooth is still under rubber dam isolation. However, post space preparation is frequently delayed and done by a restorative dentist rather than the endodontist,² who may not follow a strict aseptic preparation protocol. In this respect, the length of the remaining root canal filling, the type of sealer used and the obturation technique are factors that may influence post treatment bacterial microleakage and jeopardize endodontic success.³⁻⁵

Several studies have been conducted to analyze the effect of immediate and delayed post preparation on the performance of the remaining canal filling.⁶⁻⁹ The results of recent publications¹⁰⁻¹¹ suggest that the use of EndoREZ (ER), a urethane dimethacrylate-based sealer together with resin-coated gutta-percha cones^a (RGPC) offer a promising alternative for obturation after root canal preparation. The hydrophilic properties of the EndoRez sealer promotes penetration of the sealer into moist dentin and dentin tubules^{10,12} thus substantially reducing microleakage.¹⁰ ER has a setting time of approximately 15-20 minutes at body temperature. In a pilot experiment (Zmener & Pameijer, 2009; unpublished data), the setting time of ER was

tested in an incubator at 37°C/100% relative humidity and according to specification No. 57 of the ANSI/ADA 2000, guidelines for root canal sealing materials.¹³ The results showed a mean setting time of 19.5 minutes (range 17-21 minutes). By adding an accelerator,^a a rapid polymerization of 5-6 minutes is accomplished. This study analyzed the sealing properties of root fillings with RCGP and ER with and without the accelerator. The roots were then prepared with a post space, either immediately after completion of the endodontic treatment or after 14 days. The null hypothesis was that both immediate and delayed post-space preparation did not affect the coronal seal.

Material and Methods

For this study a total of 48 extracted human teeth with single, straight, round-shaped root canals were used. The teeth were stored in deionized water with a few crystals of thymol until being worked on. Inclusion of a tooth was determined radiographically; the apical 5-6 mm of the canal had to be round as determined on the basis of a ratio of at least 1:1 in a mesio-distal to bucco-lingual direction. The crowns were sectioned such that a standardized root length of 18 mm was created. After removal of gross pulpal tissue, the working length (WL) was established by advancing a size 10 K-file into the canal until just visible at the apex and then subtracting 1 mm. In all teeth the coronal and middle thirds were flared with #2-#3 Gates Glidden drills^b and the canals prepared to the WL with K-Type files^b using a standard push-pull circumferential filing technique. Biomechanical preparation of the apical part

of the canals was considered complete when a size 40 file could easily be inserted to the WL. The remainder of the canal was prepared with a step-back technique coronally to a size 60 file. Throughout preparation, and at each change of instrument, the canals were irrigated with 10 mL 5.25% NaOCl followed by 10 mL 17% ethylene diamine tetraacetic acid (EDTA). After preparation, the canals were profusely rinsed for 1 minute with 17% EDTA solution. Patency was confirmed with a size 10-K file when the tip protruded 1 mm beyond the apex. Excess irrigating solution was removed according to a procedure described by Zmener *et al.*,¹⁰ which kept the root canal walls moist. A luer adapter operating at low vacuum was used for 5 seconds followed by two sterile paper points (1 second each). The canal was determined moist if at least 3 mm of the last point showed moisture. Bucco-lingual and mesio-distal radiographs allowed for an assessment of gross similarities in anatomy, which formed the basis for a reasonable equitable distribution of the teeth between four experimental groups of 10 teeth each (n= 10). The grouped specimens were steam autoclaved and stored under sterile conditions in 100% relative humidity at 37°C until further use. All obturation and post space preparation procedures as well as further bacterial testing were conducted under sterile conditions in a microbiology laboratory. In all teeth, ER sealer was introduced into the canals using a #30 gauge Navitip^a according to the manufacturers' recommendations. Then, the obturation of the canals was completed and the post space preparation was performed as follows:

Group 1: (n= 10) Master RGPC with friction fit at the working length complemented with 3-5 size #25 RGPC .02 taper accessory cones. After seating of the master cone the accessory cones were "harpooned" in the EndoREZ as far as possible. Excess gutta-percha was removed with a heated instrument at the level of the coronal access. Samples were kept at 37°C and 100% relative humidity in an incubator during 20 minutes. Post space preparations were performed 2 minutes after removal from the incubator, that is about 22 minutes from the moment of filling and adding the accessory cones. The samples were kept in an incubator for another 15 minutes and then prepared for the bacterial leakage test.

Group 2: (n=10) Placement of the master cone was similar to Group 1, however, the placement of the accessory cones was different. Two to three #25 RGPC .02 taper accessory cones were dipped in the accelerator^a and harpooned into the EndoRez as far as possible. Excess gutta-percha was severed with a heated instrument at the level of the coronal access. Samples were then kept at 37°C at 100% relative humidity in an incubator for 6 minutes. Post space preparations were done 2 minutes after removal from the incubator, that is about 8 minutes from the moment of filling and adding the accessory cones. Specimens were subsequently kept in the incubator for another 15 minutes until preparation for the bacterial test.

Group 3: (n=10) Sample preparation was the same as for Group 1, however, post space preparations were performed 7 days after the root canal filling had been completed. During the 7-day interim period, a sterile cotton pellet was placed in the canal orifice and the specimens stored in an incubator at 37°C in 100% relative humidity until post space preparation. Upon completion of the post space, the specimens were kept in the incubator for another 15 minutes and then subjected to the bac-

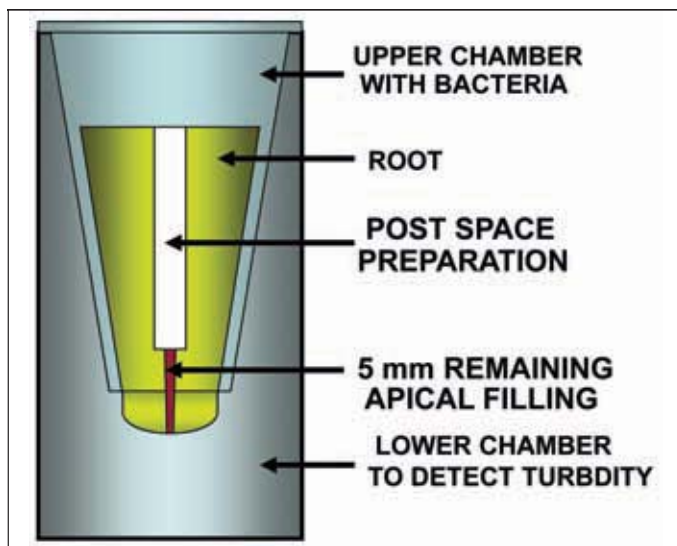


Figure. Schematic representation of the set-up for the determination of bacterial leakage.

terial leakage test.

Group 4: (n=10) This group was similar to Group 2, except the post space preparation was performed 7 days after completion of the root canal filling.

In all groups, post space preparation was initiated by removing the filling material with sterile #2 to #5 Gates Glidden drills^b to a depth of 13 mm leaving 5 mm of apical filling. Sterile size #5 (red), #5.5 (purple) and #6 (black) post space preparation drills from the Para Post System^c were then progressively used to prepare a final post space of 13 mm depth and ± 1.5 mm internal diameter. Both Gates Glidden and the Para Post drills were used in an electric motor at an approximate speed of 900 rpm. The depth was standardized with a silicone stopper set at 13 mm. Each drill was used with light apical pressure with in and out movements along with sterile distilled water-spray cooling. After use, the drills were cleaned with alcohol and reused for no more than four samples. Care was taken not to cause damage to the seal during post space preparation. To verify this step the teeth were radiographed bucco-lingually and mesio-distally to examine the quality of the apical seal. Samples judged inadequate were replaced. The teeth were then coated with two layers of nail varnish and one layer of sticky wax except for 1 mm around the coronal opening of the canals and 1 mm around the apical foramen.

One negative control specimen per group was obturated and treated as in each corresponding experimental group, except the roots were entirely varnished including the canal orifice and apex. One positive control per group was instrumented in a similar way but they were neither obturated nor varnished. All negative and positive control specimens were tested for leakage in the same way as the experimental groups.

Bacterial leakage setup - The microbial leakage model consisted of a slight modification of the dual chamber test apparatus as described by Imura *et al.*¹⁴ The tips of 1.5 ml Eppendorf plastic tubes (upper chamber) were cut and the obturated roots were pushed through the openings until they protruded approximately 6-7 mm (Figure). The junction between the tooth and the tube was sealed with sticky wax. The tubes were put into glass vials (lower chamber) containing 10

Table. Samples showing turbidity per 20-day interval.

Group	n	Period (days)			No leakage
		1 – 20	21 – 40	41 – 60	
1	10	0	1	2	7 (70%)
2	10	0	1	3	6 (60%)
3	10	1	5		4 (40%)
4	10	0	6		4 (40%)

ml of sterile trypticase soy broth^d (TSB) in such a way that approximately 4 mm of the root end was submerged in the broth. The junction between the tube and the glass vial was sealed with sticky wax. The entire test apparatus was sterilized with ethylene oxide gas for 12 hours and then incubated at 37°C for 72 hours to ensure sterility. If the TSB broth showed signs of turbidity, the test sample was discarded and replaced.

Bacterial leakage test - The upper chamber was filled with 1 ml of TSB containing 24-hour growth of *Enterococcus faecalis* ATCC 29212 (10^8 colony-forming units/ml). The inoculated apparatus was incubated for 60 days at 37°C. Based on a previous report by Torabinejad *et al*,¹⁵ the upper chamber was reinoculated every 5 days with freshly cultured microorganism. The TSB broth in the lower chamber was checked daily for turbidity, which indicated bacterial leakage through the entire length of the root canal. Once turbidity was detected, the day was recorded. Samples from both chambers were then incubated on blood-agar plates to check bacterial viability through observation of the morphology and Gram staining. The number of teeth demonstrating bacterial leakage and the day on which leakage occurred were recorded for each group. The number of teeth that had not leaked at the end of the experiment was recorded as zero leakage. The length of time until leakage was detected was statistically compared among groups using the Kaplan-Meier survival analysis. Significant pairwise differences were analyzed using the log-rank test at $P < 0.05$.

Results

Before starting the experiment, two samples in Group 1 and one sample in Group 4 showed signs of contamination. These samples were discarded and replaced. All positive controls showed bacterial leakage within 48 hours whereas none of the negative controls leaked. The results for the experimental groups and test periods are shown in Table 1.

In Group 1, turbidity did not occur until 30 days (one sample) and 42 days (two samples). In Group 2, leakage occurred at 31 (one sample), and 43 days (three samples). In Group 3, samples leaked at 14 (one sample), 21 and 28 days (two and three samples respectively). In Group 4, leakage occurred at 22 (two samples), 24 (one sample), 27 (one sample) and 28 days (two samples). All bacteriological testing of turbidity in the lower chamber demonstrated viable *E. faecalis*. No significant differences ($P > 0.05$) were detected between Groups 1 and 2 as well as between Groups 3 and 4. The results of Groups 1 and 2 differed significantly ($P < 0.05$) from Groups 3 and 4. Therefore, the null hypothesis was rejected. The Median survival time (absence of bacterial leakage) was 28 days in Groups 3 and 4 (with a 95% confidence interval of 20.9-35.1 and 26.5-29.5 respectively), whereas it could not be determined for Groups 1 and 2, since it

was greater than 60 days, the maximum time of observation.

Discussion

Materials that are used to obturate the root canal space have to be able to establish a tight seal at the interface filling material/root canal walls. Preparing an obturated canal for a post removes a substantial amount of the root filling and may disturb the seal of the remaining apical filling.^{16,17} In this study, we analyzed coronal bacterial leakage of root fillings with RGPC and ER sealer in root canals that had a post space prepared either immediately after completion of the root canal treatment or after 7 days. The same protocol was followed for two additional groups in which an accelerator was used to reduce the setting time of ER. An evaluation was done of the number of samples with coronal leakage of *E. faecalis* and the length of time (1 to 60 days) needed for the microorganisms to penetrate through the apical filling material that remained after post space preparation. *E. faecalis* was chosen because they are part of the normal oral flora in humans and are frequently found in mixed infections with other aerobes and facultative anaerobes. Moreover, *E. faecalis* is one of the most commonly isolated bacteria from infected root canals.¹⁸ The parameters used for evaluation were qualitative (presence or absence of turbidity) not quantitative. In spite of the fact that a 60-day bacterial penetration test *in vitro* as reported here, may not be an exact imitation of the events that take place clinically, it allows for comparison between groups under strict controlled conditions.

The length of remaining apical obturation and the time at which the post space was prepared, as well as their relationship with the length of time required by the microorganisms to penetrate through the apical filling material are important variables that need to be discussed; for instance, the remaining 5 mm of filling material that was chosen in this study. A minimum of 5 mm is commonly recommended to avoid compromising the integrity of the apical seal.^{19,20} Previous investigations²¹⁻²⁵ have demonstrated that an apical filling of less than 4 mm showed a statistically significant increase in leakage. Additional *in vivo* observations by Kvist *et al*,²⁶ found that teeth with a post in which the remaining root canal filling was less than 3 mm revealed a significantly higher frequency of periradicular radiolucencies than teeth that had longer root canal fillings. These observations were supported by De Cleen¹ who suggested that ± 6 mm length of the root filling should be left in place.

In a pilot study (unpublished data) teeth with intact root canal fillings of either RCGP/ER or RCGP/ER/Accelerator without post space preparation, were subjected to coronal bacterial leakage. Bacterial penetration was observed in a few specimens after 54-57 days with no significant differences between the two groups. In the experiment reported here, however, bacterial penetration through the remaining apical filling was observed much sooner, whether the accelerator was used or not. These results were not totally surprising. The difference may be due to either the post space preparation disturbing the seal of the remaining apical filling or because the post space preparation significantly shortened the distance from the canal opening to the remaining apical filling, thereby reducing the time the bacteria needed to reach the apical foramen, in addition to increasing the amount of penetrating microorganisms. These results are in agreement with the study

of Abramovitz *et al*²⁷ who reported that an apical root filling of 5 mm provided an inferior seal compared to a full length root canal filling.

The post space preparation was initiated with Gates-Glidden drills to remove the coronal 8 mm of the root fill followed by shaping with post space drills. The results demonstrated that the effect of rotary instruments on the apical seal is minor, while other methods such as the use of chemical solvents or heated instruments are less desirable.^{1,24} Gutta-percha removal using progressively larger diameters of Gates Glidden drills appears to be a safe procedure. As demonstrated by Gegauff *et al*,²⁸ large deviations from the main canal were produced when only one post space drill was used.

Whether an accelerator was used or not, specimens in which the post space preparation was delayed (Groups 3 and 4) leaked significantly more than the group that was prepared immediately (Groups 1 and 2). Furthermore, there were no significant differences in coronal bacterial leakage between Groups 3 and 4 or between Groups 1 and 2. These results contradict Cobankara *et al*,²⁹ who found significant differences between groups with and without the accelerator when the post space preparation was prepared immediately. The authors suggested that the accelerator did not offer clinicians the advantage of completing endodontic treatment followed by an immediate post-endodontic restorative procedure.²⁹ Our findings that delayed post space preparation resulted in more leakage are in agreement with the results of previous experiments,^{7,8,22,30} in which different types of non-methacrylate-based sealers were used along with gutta-percha for filling root canals. However, they differ from Lyons *et al*³¹ and Bodrumlu *et al*,⁹ who also used a methacrylate-based resin material for obturation (Resilon/Epiphany) and found more leakage in teeth in which the post space preparation was done immediately after endodontic treatment *versus* 7 days later.

Why more bacterial leakage occurred in the delayed post space preparation groups defies an easy explanation. In a Field Emission Electron Microscope (FESEM) study, Bergmans *et al*¹² postulated that the high viscosity and hydrophilic nature of ER allowed for easy penetration of the sealer in moist dentin tubules but that after polymerization, the shrinkage-related stresses gradually increased leading to disruption of the resin tags formed between the core material and dentin, which over time leads to the formation of gaps.¹² Based on the above we can speculate that the results of Groups 3 and 4, in which the post space preparation was done 7 days after the canal filling, may have occurred as a result of the post space preparation. This may have caused damage to the resin tags causing a disruption of the bond of the sealer/dentin interface of the 5 mm remaining apical filling. *Vice versa*, when preparing a post space preparation immediately after the ER sealer has set, at the moment that the polymerization shrinkage stresses have not been fully developed, challenges a remaining apical filling that is better able to resist the effect of rotary instruments.

It should be stressed that, although in this study the differences in leakage between immediate and delayed post space preparation were statistically significant, it may not be clinically significant. This is because clinically acceptable rates of leakage have not been determined yet.

In conclusion, delayed post space preparation (7 days) re-

sulted in a more pronounced and accelerated coronal bacterial leakage compared to post preparations that are done immediately after setting of ER. It was also demonstrated that the use of the accelerator *per sé* was not a factor that influenced leakage. This is an important issue since many clinicians do not use aseptic techniques and rubber dam isolation during delayed post space preparation and post cementation. Consequently, in the absence of an aseptic technique, contamination may occur, which can further jeopardize the outcome of the endodontic treatment. It is therefore recommended that in the event that ER sealer is used, a post space is prepared immediately after the sealer has set. Using the accelerator will shorten the setting time of the sealer thus allowing the practitioner to prepare the post space during the same session the endodontic treatment is completed.

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- b. Dentsply/Maillefer, Ballaigues, Switzerland.
- c. Coltene/Whaledent Int, Cuyahoga Falls, OH, USA.
- d. Difco Laboratory, Detroit, MI, USA.

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