

# Effect of Timing and Method of Post Space Preparation on Sealing Ability of Remaining Root Filling Material: In Vitro Microbiological Study

*Fabiana Soares Grecca, DDS, MSc, ScD; Ângela Rezende Gomes Rosa, DDS; Maximiliano Schünke Gomes, DDS, MSc; Clarissa Fatturi Parolo, DDS, MSc, ScD; Jules Renan Dutra Bemfica; Luis Carlos da Fontoura Frasca, DDS, MSc, ScD; Marisa Maltz, DDS, MSc, ScD*

## Contact Author

*Dr. Grecca*  
Email: [fakera@terra.com.br](mailto:fakera@terra.com.br)



## ABSTRACT

**Objective:** To evaluate the effect of timing (immediate versus delayed) and technique of post space preparation on the ability of the residual root canal obturation to prevent coronal bacterial leakage.

**Materials and Methods:** Sixty-six single-rooted teeth were decoronated at the cemento-enamel junction. The canals were prepared according to a step-back technique and were filled with thermoplasticized gutta-percha and AH Plus endodontic sealer (Dentsply De Trey). The root segments were randomly assigned to 8 groups. The positive controls ( $n = 3$ ) were instrumented but not obturated. The negative controls ( $n = 3$ ) were instrumented, obturated and sealed with Cavit (3M ESPE). In the other 6 groups ( $n = 10$  each), the post space was prepared either immediately after obturation or 7 days later using LA Axxess burs (SybronEndo) (groups 1 and 2), heated pluggers (groups 3 and 4) or solvent delivered with a hand file (groups 5 and 6). The external surface of all roots was rendered waterproof with nail varnish. Custom-made dual-chamber devices were used to evaluate leakage. The coronal third of the prepared root canal was kept in contact with artificial saliva contaminated with *Enterococcus faecalis*, and the root apex was submerged in tryptic soy agar medium. The root assemblies were stored at 37 °C and were monitored daily over a 90-day period. The occurrence of turbidity in the medium was deemed to indicate bacterial leakage, from which failure of the seal was inferred.

**Results:** Throughout the experimental period, there was no significant difference ( $p = 0.094$ ) among the preparation techniques, either immediate or delayed, in terms of bacterial leakage.

**Conclusions:** Immediate and delayed post space preparation yielded similar outcomes in terms of the canal seal. Regardless of the timing and the technique of post space preparation, coronal bacterial leakage occurred over time.

For citation purposes, the electronic version is the definitive version of this article: [www.cda-adc.ca/jcda/vol-75/issue-8/583.html](http://www.cda-adc.ca/jcda/vol-75/issue-8/583.html)

Endodontic treatment is a common step in prosthetic rehabilitation. It is indicated not only in cases of pulpal alteration, but also when intraradicular retainers are required.<sup>1</sup> The procedures for preparing the post

space are critical, and care is required to maintain the root canal seal and the aseptic conditions achieved with endodontics. Otherwise, a pathway may be created for bacterial invasion and re-infection of the root canal system.

Microorganisms and their byproducts are the primary cause of pulpal and periapical pathosis.<sup>2</sup> In particular, *Enterococcus faecalis* can penetrate dentinal tubules quickly and deeply, is resistant to intracanal medications and has been associated with refractory cases of such problems.<sup>3,4</sup>

Although the creation of a hermetic 3-dimensional seal in endodontically treated teeth remains a challenge, the residual root canal obturation should, in theory, act as a physical barrier to prevent fluid and microbial leakage that might lead to endodontic failure.

The procedures for preparing the post space are therefore critical, and care is required to maintain the root canal seal and the aseptic conditions achieved with endodontics.<sup>5,6</sup> Depending on the technique (mechanical [burs/drills], physical [heated instruments] or chemical [solvents]<sup>1,5,7</sup>) and the timing (either immediately after obturation of the root canal or at some later time<sup>7-9</sup>), the residual filling material may become displaced, which creates a pathway for bacterial invasion and re-infection of the root canal system.

The purpose of this study was to evaluate the effect, over a 90-day experimental period, of timing (immediate versus delayed) and technique (burs, pluggers or solvent) of post space preparation on the sealing ability of the remaining root filling material against coronal leakage of *Enterococcus faecalis*. The null hypothesis was that the timing and method of post space preparation would have no influence on the sealing ability of the remaining apical filling.

## Materials and Methods

This study was approved by the Ethics in Research Committee of the Federal University of Rio Grande do Sul.

Sixty-six extracted human single-rooted teeth with straight roots and closed apices were randomly selected and checked for absence of root caries, cracks and structural defects. The crown of each tooth was removed at the cemento-enamel junction, and the roots were stored in buffered 10% formalin solution until use.

The root canal of each specimen was irrigated with 1% sodium chloride (NaOCl) and explored with a size 10 K-file (Dentsply/Maillefer, Ballaigues, Switzerland). For each specimen, the file was introduced into the canal and advanced until its tip could be seen through the apical foramen. The working length was defined as 1 mm less than the length of the root. The teeth were prepared with a hand instrumentation technique using size 15 to 50 K-files. The last instrument for each tooth was the size 50 K-file, to standardize the enlargement of the canals. The root canals were irrigated with 2 mL of 1% NaOCl at each change of file size, followed by aspiration and drying with absorbent paper points (Tanari, Manaus, Brazil).



**Figure 1:** Example of radiograph used to evaluate the quality of the root filling, specifically, homogeneity and apical extension.

Three root segments were randomly selected for the positive control group (root canals that were instrumented but not obturated). The remaining roots were filled according to Tagger's hybrid technique with AH Plus endodontic sealer (Dentsply De Trey GmbH, Konstanz, Germany) and #50 principal gutta-percha cone and fine to medium accessory gutta-percha cones (Dentsply De Trey) for obturation. Buccolingual and mesiodistal radiographs were obtained to evaluate the quality of the root filling, specifically homogeneity and apical extension. If voids were detected, additional accessory gutta-percha cones were inserted using the lateral condensation method and Tagger's hybrid technique to ensure that the fillings were well compacted. Excess material was removed by application of a heated plugger at the coronal level, and the remaining filling material was vertically condensed using Schilder pluggers 3 and 4 (Dentsply Maillefer) (**Fig. 1**). At this point, 3 roots were randomly selected for the negative control group (root canals that were obturated but did not undergo post preparation).

The post space was prepared immediately after the root canal obturation or 7 days later using burs (groups 1 and 2, respectively), heated pluggers (groups 3 and 4, respectively) or solvent delivered with a hand file (groups 5 and 6, respectively) (**Table 1**). The external surface of all roots was rendered waterproof with nail varnish. In all experimental groups, the root fillings were reduced to a length of 4 mm in the apical third. The roots scheduled for delayed post space preparation (groups 2, 4 and 6) were stored for 7 days at 100% relative humidity at 37 °C to allow for complete setting of the sealer.

For roots in groups 1 and 2, a rotating LA Axxess bur (SybronEndo, Glendora, CA) coupled to a low-speed

**Table 1** Experimental groups

Group	n	Post space preparation	Timing of post space preparation
1	10	LA Axxess	Immediately
2	10	LA Axxess	7 days later
3	10 <sup>a</sup>	Heated pluggers	Immediately
4	10	Heated pluggers	7 days later
5	10	Solvent	Immediately
6	10 <sup>a</sup>	Solvent	7 days later

<sup>a</sup>One sample discarded because of handling problems during bacterial inoculation.

handpiece was introduced into the canal to the predetermined length. The bur was inserted and withdrawn several times with rotation until the filling material had been completely removed from the canal walls. For groups 3 and 4, the post space was created using heated size 2 and 3 Rhein root canal pluggers (Golgran Ind., São Paulo, Brazil) inserted to the predetermined length. For groups 5 and 6, the solvent xylol was delivered with a K-file to the predetermined length.

Vertical force was applied with cold Schilder pluggers to compact the remaining root filling mass. Excess sealer was removed from the canal walls with cotton pellets soaked in alcohol. Cleanness of the canal walls was confirmed radiographically.

The obturated root segments were sterilized with ethylene oxide.

To determine the presence of bacterial leakage, a dual-chamber device similar to the one designed by Torabinejad and colleagues<sup>10</sup> was fabricated for each root.

*Enterococcus faecalis* (ATCC 29212) was used for the bacterial leakage test. The bacteria were maintained in tryptic soy agar (TSA, Merck KGaA, Darmstadt, Germany) at 4 °C. The strain was subcultured weekly in the same medium. A loop of the *E. faecalis* strain retrieved from this medium was diluted in 10 mL of saline. The concentration of the microbial suspension was adjusted to achieve a turbidity equivalent to tube 1 of the McFarland standard (Bio Mérieux, Marcy L'Étoile, France), to ensure a bacterial concentration of about 3 × 10<sup>8</sup> colony-forming units per millilitre, corresponding to an optical density between 0.036 and 550 nm, as measured with a spectrophotometer (Spectronic 21D, Milton Roy Company, Rochester, NY). This wavelength was used to standardize the amount of inoculum at each change of the culture broth.

About 1 mL of a mixture of bacterial suspension and sterile artificial saliva (ratio 3:1) was added to each Eppendorf tube, which was then sealed. The glass vials containing the Eppendorf tube and root assemblies were stored at 37 °C for 90 days. To ensure the viability of the biological tracer, the microorganism–TSA–saliva

mixtures were renewed weekly during the experimental period. The mixture in each Eppendorf tube was aspirated with a sterile disposable needle and syringe and discarded, and was immediately replaced with freshly prepared mixture. To confirm the sterility of the culture medium used in the experiment, a sample was placed in a covered glass vial and visually monitored daily.

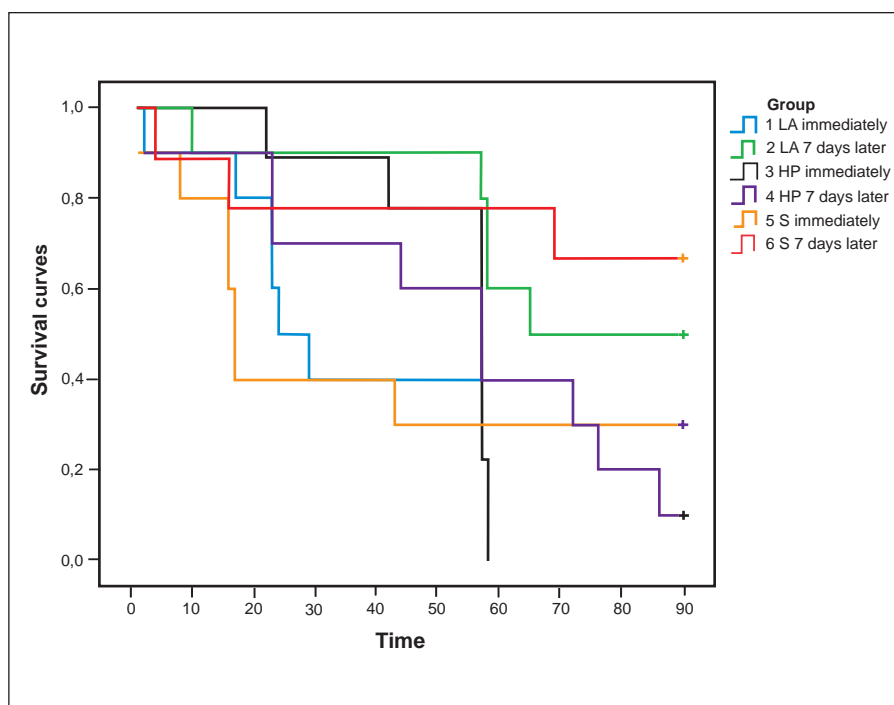
Over the 90-day experimental period, the culture medium in the lower chamber of each device was checked daily by a calibrated examiner who was blinded to the group assignment of each root. The presence of turbidity in the medium in which the apices of the roots inoculated with *E. faecalis* were submerged was deemed to indicate that bacteria had penetrated through the residual filling material and reached the medium. The time elapsed (in days) until the occurrence of turbidity was recorded for each sample, at which point the sample was discarded. Lack of turbidity of the medium indicated no bacterial leakage.

The Kaplan–Meier method was used to estimate survival curves for each experimental group, with “death” of a specimen deemed to have occurred at the time when bacterial turbidity was first observed. Log-rank testing was used to compare the survival curves at the 5% significance level.

## Results

The positive controls exhibited bacterial leakage after 1 day of inoculation, whereas no leakage was observed for the negative controls over the entire 90-day period. The control sample of the culture medium showed no turbidity, which indicated that no bacterial growth occurred. One sample from group 3 (prepared by heated plugger immediately after obturation) and another from group 6 (prepared by solvent after 7-day delay) were discarded because of handling problems during bacterial inoculation.

Throughout the experimental period, there was no statistically significant difference ( $p = 0.094$ ) among the preparation techniques when performed immediately or after a 7-day delay (Fig. 2, Tables 2–4).



**Figure 2:** Kaplan–Meier survival curves for bacterial leakage in the experimental groups over the 90-day period. LA = LA Axxess burs, HP = heated pluggers, S = solvent.

## Discussion

In the study reported here, there was no significant difference in the sealing ability of the remaining root filling material related to the technique or the timing of post space preparation, consistent with the findings of previous studies.<sup>8,9</sup> The null hypothesis was therefore rejected.

However, in the first 20 days, maintenance of the seal was excellent for the roots for which heated pluggers were used immediately after obturation (group 3), as none of these specimens presented any bacterial leakage (turbidity of medium) during this period (**Table 2**). In clinical terms, this period corresponds to the time required for post space preparation and cementation of the intraradicular retainer. Attention to the adaptation and appropriate sealing of temporary elements that form the foundation of the intraradicular retainer and the subsequent definitive restoration are clinically important. If the post space is prepared immediately, it can be done by the endodontist, which facilitates maintenance of the aseptic chain.

In a previous study,<sup>7</sup> the root canals were filled with the same sealer, using the same technique, as in this study, and significantly more dye leakage occurred when the post space was prepared 1 week after obturation, relative to preparation immediately after obturation. The authors of that study hypothesized that when post space preparation is delayed, the sealer sets completely, such that removal of the filling mass later on causes move-

ment that disrupts the bond at the sealer interface.

In the study reported here, LA Axxess rotary instruments were used for post space preparation, but no leakage studies involving this instrument were found in a search of the literature. Although some authors have reported that the use of rotary instruments does not disrupt the integrity of the apical seal, Haddix and colleagues<sup>11</sup> found significantly greater leakage after removal of gutta-percha with Gates Glidden drills and GPX instruments than after removal with heated pluggers.

Another factor that may jeopardize the apical seal during post space preparation is the length of the residual filling material. The amount of gutta-percha left in the apex in the current study was in accordance with several previous reports,<sup>7,12-14</sup> in which the authors advocated that a minimum of

4 mm of filling material should remain in the root canal to prevent bacterial leakage. In addition, the length of the remaining filling was very close to the length that is clinically accepted as the minimal limit of apical obturation in endodontically treated teeth scheduled for placement of an intraradicular retainer.<sup>11</sup>

In the present study, the durability of canal sealing was similar, regardless of technique and regardless of the timing of post space preparation. Monitoring of the prepared teeth over a period of 90 days showed that, regardless of the technique, the remaining filling material did not prevent coronal leakage of *E. faecalis*.

The 90-day experimental period was based on the methods in previous coronal leakage studies.<sup>12-14</sup> However, dental practitioners should also be aware of the possibility of bacterial invasion through obturated root canals in a shorter time (20 to 50 days), as observed in this study and reported elsewhere.<sup>4,10</sup> These findings confirm that the presence of residual root canal obturation delays but cannot prevent bacterial invasion within root canals exposed to the oral environment. The more quickly the prosthetic rehabilitation of endodontically treated teeth is completed, the smaller the chances of failure of the canal seal.

Various in vitro methodologies, such as the use of radioisotopes, dye and pressure-driven fluid systems, have been used to evaluate the seal of root-filled teeth, but the results have been inconsistent.<sup>8,9,12,15,16</sup> However,

**Table 2** Frequency of bacterial leakage by study group, over the 90-day experimental period

Group	Time of assessment; no of specimens with bacterial leakage								
	Day 10	Day 20	Day 30	Day 40	Day 50	Day 60	Day 70	Day 80	Day 90
1. LA Axxess, immediate ( <i>n</i> = 10)	1	2	6	6	6	7	7	7	7
2. LA Axxess, delayed ( <i>n</i> = 10)	1	1	1	1	1	4	5	5	5
3. Heated pluggers, immediate ( <i>n</i> = 9)	0	0	1	1	2	9	9	9	9
4. Heated pluggers, delayed ( <i>n</i> = 10)	1	1	3	3	4	6	6	8	9
5. Solvent, immediate ( <i>n</i> = 10)	2	6	6	6	7	7	7	7	7
6. Solvent, delayed ( <i>n</i> = 9)	1	2	2	2	2	2	3	3	3

**Table 3** Frequency of bacterial leakage according to type of post space preparation, over the 90-day experimental period

Method of preparation	Time of assessment; no. of specimens with bacterial leakage								
	Day 10	Day 20	Day 30	Day 40	Day 50	Day 60	Day 70	Day 80	Day 90
LA Axxess ( <i>n</i> = 20)	2	3	7	7	7	11	12	12	12
Heated pluggers ( <i>n</i> = 19)	1	1	4	4	6	15	15	17	18
Manual ( <i>n</i> = 19)	3	8	8	8	9	9	10	10	10

**Table 4** Frequency of bacterial leakage according to timing of post space preparation, over the 90-day experimental period

Timing of preparation	Time of assessment; no. of specimens with bacterial leakage								
	Day 10	Day 20	Day 30	Day 40	Day 50	Day 60	Day 70	Day 80	Day 90
Immediate ( <i>n</i> = 29)	3	8	13	13	15	23	23	23	23
Delayed ( <i>n</i> = 29)	3	4	6	6	7	12	14	16	17

all of these studies have indicated some extent of coronal leakage. Among the in vitro methods that have been used, testing for bacterial leakage reflects clinical reality, since it uses a biological marker.

Several bacterial species have been used to assess leakage in obturated root canals.<sup>10,13,15,16</sup> Because of its capacity to invade dentinal tubules, compete with other microorganisms and resist nutritional privation, *E. faecalis* is the pathogen most frequently isolated from endodontic infections. The choice of *E. faecalis* for this

study was based on its bacteriological profile and its ease of handling in in vitro assays.<sup>3,4</sup>

Endodontic sealers are used to fill the interface between the gutta-percha and the root canal walls. AH Plus is an epoxy resin-based sealer with wide clinical acceptance. This material has good physicochemical properties, including sealability and dimensional stability, and an 8-hour setting time.<sup>4,7,17,18</sup>

The obturated root segments were sterilized with ethylene oxide because it has been demonstrated that

this process does not alter the permeability of the dentin.<sup>19</sup>

The absence of turbidity of the medium with the negative controls confirmed the suitability of the dual-chamber device for this study. The presence of turbidity of the medium with the positive controls showed that bacterial leakage occurred through the root canal extension, confirming that microorganisms might reach the apical region in the absence of a seal.

The findings of the study reported here confirm that the presence of residual root canal obturation delays but cannot prevent bacterial invasion into root canals exposed to the oral environment over time, if the coronal seal is defective. The more quickly prosthetic rehabilitation of endodontically treated teeth is completed, the smaller the chances of failure of the canal seal, independent of the technique and the timing of post space preparation. ✦

## THE AUTHORS



**Dr. Grecca** is adjunct professor in the discipline of integrated clinic, School of Dentistry, Federal University of Rio Grande do Sul, Porto Alegre, Brazil.



**Dr. Rosa** is in private practice in Rio Grande do Sul, Porto Alegre, Brazil.



**Dr. Gomes** is captain, Medical and Dental Centre, Health Department of the Military Brigade, Porto Alegre, Brazil.



**Dr. Parolo** is assistant professor in the department of preventive and community dentistry, School of Dentistry, Federal University of Rio Grande do Sul, Porto Alegre, Brazil.



**Mr. Bemfica** is an undergraduate student in the School of Dentistry, Federal University of Rio Grande do Sul, Porto Alegre, Brazil.



**Dr. Frasca** is adjunct professor in the discipline of integrated clinic, School of Dentistry, Federal University of Rio Grande do Sul, Porto Alegre, Brazil.



**Dr. Maltz** is professor and chair of the department of preventive and community dentistry, School of Dentistry, Federal University of Rio Grande do Sul, Porto Alegre, Brazil.

**Acknowledgements:** The authors acknowledge Professor Gertrudes Corção of the Laboratory of Microbiology at the Institute of Biosciences,

Federal University of Rio Grande do Sul, Brazil, for kindly donating the *Enterococcus faecalis* ATCC 29212 strain used for bacterial testing.

**Correspondence to:** Dr. Fabiana Soares Grecca, Universidade Federal do Rio Grande do Sul, Faculdade de Odontologia, Rua Ramiro Barcelos, 2492, Centro, 90035-003 Porto Alegre, RS, Brazil.

The authors have no declared financial interests in any company manufacturing the types of products mentioned in this article.

This article has been peer reviewed.

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