Efficacy of intracanal barrier against microbial infiltration in teeth prepared for intraradicular post

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ABSTRACT

Introduction: Intraradicular posts are recommended to improve the retention of artificial crowns and to distribute intraoral forces along the root. If the space created by partially removing the obturation is not adequately filled, it can lead to a massive infiltration of microorganisms from the oral cavity. **Aim:** The aim of this study was to assess the influence on bacterial infiltration of an intracanal barrier placed directly over the remaining root canal filling following post space preparation. **Material and Methods:** Seventy-two human single-rooted teeth were instrumented, filled, and then randomly divided into three experimental groups and two control groups. Group 1 received no additional treatment after filling and post space preparation, whereas Groups 2 and 3 received a barrier composed of temporary sealing material 1.0- and 2.0-mm thick, respectively. A culture of *Enterococcus faecalis* was inoculated into the spaces prepared to receive the intraradicular post, every three days over a 60-day period. Infiltration was evaluated daily by observing the turbidity of the culture medium. **Results and Conclusion:** Bacterial infiltration was found in all three experimental groups, but was significantly greater and occurred more quickly in G1 ($p < 0.05$), compared with G2 and G3. Infiltration was observed in all positive controls, whereas no infiltration was found in the negative controls. No significant difference ($p > 0.05$) was found between G2 and G3, regarding the rate and time frame of infiltration. It can be concluded that the barrier reduced the incidence of infiltration, and delayed the time of occurrence.

Keywords: Dental Pins. Endodontics. Enterococcus faecalis.

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Introduction

Cleaning, conical preparation, and hermetic and three-dimensional filling of the root canal system (RCS) are factors that determine successful endodontic treatment.[1] However, therapeutic success does not depend on these factors alone in the long term. A restoration that promotes good coronal sealing is a factor found to be important in achieving and maintaining successful endodontic therapy.[2] Root canal exposure to oral microbiota may put the treatment outcome at risk, if the endodontically treated teeth receive neither adequate coronal sealing between treatment sessions, nor, and most importantly, a restoration capable of preventing coronal infiltration.[3-7]

The role of microorganisms in the aetiology and maintenance of pulpal and periapical pathologies has been well established since 1965.[8] Therefore, it is of crucial importance that endodontic therapy not only be carried out under an aseptic filling process, but also prevent reinfection of the root canal system. This is particularly relevant for teeth in which a great part of the root canal filling material has been removed to make space for an intraradicular post. If not adequately filled, the space created by partially removing the obturation can allow massive infiltration of microorganisms from the oral cavity, and consequent proliferation. Several studies[9-20] have demonstrated that root canals only partially filled to allow placement of an intraradicular post showed poor sealing capability, compared with completely filled canals.

Valadares et al.[21] evaluated the efficacy of temporary sealing materials as protective barriers of endodontic filling material to prevent coronal infiltration of fluids, microorganisms and their by-products from the oral cavity. To the best of our knowledge, this is the first study that assesses the use of protective barriers directly applied to the apical filling material in post-prepared canals in human teeth (ex-vivo). In view of the scarcity of studies using protective barriers, the present study aimed at assessing how a Cavit-TM barrier placed directly over a 4.0-mm root filling remnant following immediate intraradicular preparation would influence an *Enterococcus faecalis* culture.

Material and Methods

After approval by the local research ethics committee, 72 human single-rooted teeth were obtained from the Pontifical Catholic University of Minas Gerais for use in the present study. The crowns were removed, so that all the roots could have a standardized length of 15 mm from cervical ridge to root apex. The specimens were stored in a 0.5% sodium hypochlorite solution (NaOCl) (Lenza Farmacêutica, Belo Horizonte, Brazil).

A #15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was introduced into the canal until its tip could be seen at the apical foramen opening. The working length (WL) was obtained by subtracting 1 mm from this measurement. The root canals were prepared by using rotary nickel-titanium instrumentation (ProTaper system, Dentsply Maillefer, Ballaigues, Switzerland) associated to manual instrumentation with K-type files and Gates-Glidden drills (Dentsply Maillefer, Ballaigues, Switzerland). The chemical-mechanical preparation was completed with ProTaper F4. At the end of the preparation, the foramen of all the specimens was cleaned with a #30 K-file to allow better standardization and to remove anatomical interferences in the foraminal region. Abundant irrigation was performed with 2 ml of 5.25% sodium hypochlorite solution at every change of instrument during instrumentation. After concluding the instrumentation, the root canals were irrigated first with 3 ml of 17% EDTA (Lenza Farmacêutica, Belo Horizonte, Brazil) for three minutes, and then with 2 ml of 5.25% sodium hypochlorite solution, before being dried with absorbent paper points (Dentsply Maillefer, Ballaigues, Switzerland).

The RCS was filled with thermoplastic gutta-percha, according to the vertical condensation technique. A size F4 gutta-percha point for the ProTaper system (Dentsply Maillefer, Ballaigues, Switzerland) was adapted to the WL, and then checked radiographically. Pulp canal sealer EWT (Kerr Sybron Endo, Glendora, USA) was used as filling cement. The cement filling was inserted with #15 K-type hand files, and the cement-covered cone was then placed inside the root canal. During the root canal filling, a space for an intraradicular post was opened by gradually removing the gutta-percha with a fine, medium-heated tip mounted onto a System B device (Analytic Technology, Redmond, USA). Vertical condensation was achieved using Schilder-type condensers (Odous, Belo Horizonte, Brazil) in descending order (sizes 4,

3, and 2). Next, a new radiograph was taken to evaluate the filling quality, which was considered adequate when the gutta-percha mass was homogeneous, with no voids detected by radiographs.

The samples were divided into three experimental groups with 20 specimens in each, and into two control groups, positive and negative, with 6 specimens in each. In G1, the filling of specimens and the preparing of post space were performed simultaneously, so that a 4.0-mm filling remnant could be left in the root canal. No barrier was placed over the filling remnant. In G2, the specimens were filled in the same way as G1, but Cavit temporary filing material (3M ESPE, Seefeld, Germany), 1.0-mm thick, was placed directly over the 4-mm filling remnant to serve as a barrier (Figure 1A). This barrier was inserted into the root canal using a Rhein probe (Odous, Belo Horizonte, Brazil), and guided to the apical filling remnant with a slightly moistened, small cotton pellet wrapped around a #35 K-file (Dentsply Maillefer, Ballaigues, Switzerland). Schilder condensers (Odous, Belo Horizonte, Brazil) were used in descending order (sizes 3 and 2) to achieve better barrier compaction. In G3, a larger barrier (2-mm thick) using the same temporary filing material was inserted into the root canal (Figure 1B), in a way similar to G2. Silicon courses adapted to #2 or #3 condensers were used to confirm the correct barrier thickness, followed by radiographic examination.

After barrier insertion, a small cotton pellet moistened with distilled water was inserted into the root canals of experimental groups G2 and G3, and was left there for 24 hours to ensure sealer setting.

The test apparatus for the dual-chamber experimental model was composed of a structure with 10-mL-glass vials (Wheaton d, Brazil), 20-mm-diameter rubber caps (Fábrica Artefatos Borracha Adnaloy SA, Brazil), and 1.5-mL Eppendorf tubes (Cral Artigos para Laboratório Ltda., Brazil). The caps were perforated in the center with a steel drill (Indústria e Comércio Graziano Ltda., Brazil) to establish an opening 11 mm in diameter. A portion measuring 7 mm was sectioned from the tubes at the tip. The Eppendorf tube was then heated and inserted inside the central perforation of the cap, under pressure for a better fit.

Impermeabilization of the specimens was achieved by coating with two layers of cyanoacrylate (Super Bonder, Henkel Loctite Ltda., Itapevi, Brazil) and one layer of nail polish, except for 3-mm apical, at one-hour intervals between applications, and were allowed to dry. The Eppendorf tube was then sealed with a layer of epoxy resin (Durepox, Alba Química Indústria e Comércio, Brazil), over which another layer of cyanoacrylate and a coat of nail polish were applied to ensure adequate impermeabilization, and the best possible sealing at the junction between the Eppendorf tube and the specimen. Positive control specimens received impermeabilization similar to those of the experimental groups, whereas

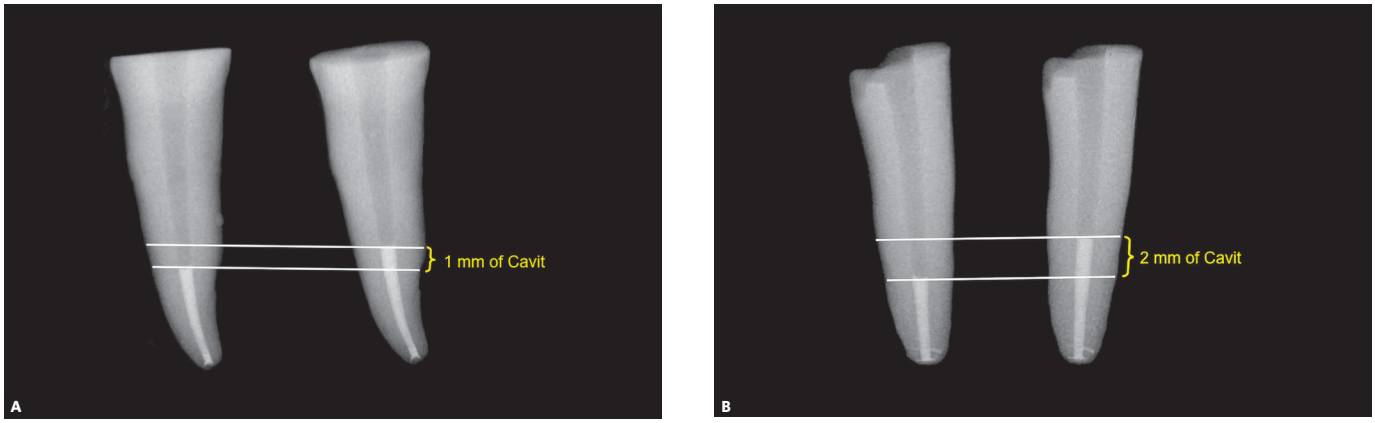

Figure 1A. G1, no barrier - G2, 1-mm barrier. **B**) G1, no barrier - G3, 2-mm barrier.

the negative control was rendered completely impermeable, including the apical 3-mm region. The sealing agents in the negative control specimens were allowed to dry for at least 24 hours at a room temperature of 24ºC, and then the entire test apparatus was duly identified and sterilized using ethylene oxide gas.

The attachment platform was assembled, and the culture medium was distributed into glass vials in a laminar flow chamber under aseptic conditions. A 6.5 mL aliquot of brain heart infusion (BHI) culture media (Difco Laboratories, Detroit, USA) was added to each glass vial, and the perforated cap was attached to the vial. Next, the Eppendorf tube-tooth assembly was inserted until approximately 3 mm of the root apex was immersed in BHI broth. The upper chamber of the apparatus was inoculated with 0.1 mL of a fresh suspension of *Enterococcus faecalis* ATCC 29212, containing approximately 3 x 10^8 cells/mL, with turbidity corresponding to 1 on the McFarland scale.

The culture was incubated for 60 days in a bacteriological incubator at 37°C, under aerobic conditions, and reinoculated every three days with a fresh suspension of microbes. The BHI broth was monitored daily, and turbidity was an indication of the presence of microorganisms in the apical segment of the root canal (Fig 2). The morphotinctorial characteristics were assessed by using both the Gram stain method and colony growth in BHI agar. The prevalence of positive samples was assessed by using Fisher's exact test at a significance level of 5% ($p < 0.05$).

The data were assessed using survival analysis. The Kaplan-Meier estimator was used to estimate the survival curve in the groups. Direct application of the Kaplan-Meier curve indicates the estimated survival probability for a given time. The Log-Rank test was employed to compare the survival curves.

Results

The results showed infiltration of all the specimens of the positive control group, whereas no specimens of the negative control group had turbidity in the culture medium during the whole experimental period. In G1, infiltration was found in 19 of the 20 specimens that had no protective barrier, whereas it was found in 7 of the 20 specimens of G2, which had a 1.0-mm-thick Cavit barrier, and in only 3 of the 20 specimens of G3, with a 2.0-mm-thick barrier.

Figure 3 shows a graphic representation of the Kaplan-Meier curves for the three groups studied. As observed, G2 and G3 performed better than G1. A statistically significant difference ($p < 0.05$) was observed when comparing G1 with G2 and G3. However, no statistical difference was found between the two latter groups ($p > 0.05$).

Figure 2. Test apparatus for dual-chamber experimental model.

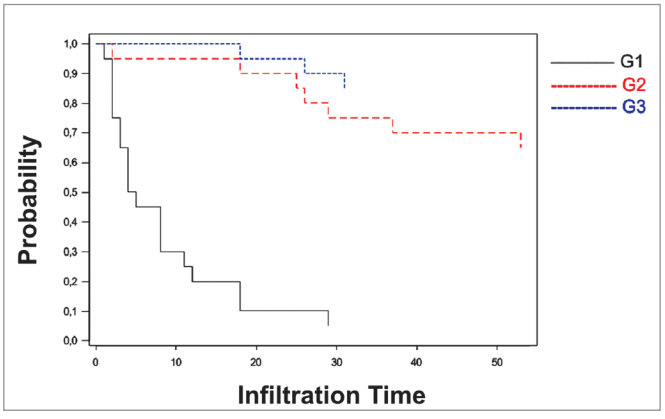

Figure 3. Graphic representation of the Kaplan-Meier curves for the three groups studied.

Discussion

Several studies have reported that coronal sealing after filling the root canal system is a fundamental step for successful endodontic therapy.[18-20,22-27] Indeed, this step becomes even more important when the root canal filling has been partially removed for post space preparation. According to Torabinejad et al.[25] and Trope et al.[28], even those teeth with completely filled root canals allow infiltration when exposed to bacteria or endotoxins present in the oral cavity. Other authors found that a successful endodontic therapy is directly related to how well done and well adapted the restorations are when concluded.[4,6,7]

According to Abramovitz et al,[13] the filling remnant should provide a seal capable of preventing coronal infiltration after post space preparation. However, loss of sealing capability in this filling remnant was observed in our study, compared to complete obturation. Metzger et al.[12] made a correlation between the remaining length of the root canal filling and the coronal infiltration. In their study, the 3-, 5-, and 7-mm filling remnants were not sealed as well as the 9-mm remnants and the 14-mm intact fillings. The results led to the conclusion that sealing capability is proportional to the length of the root filling remnant. These findings are corroborated by studies showing that 3- to 5-mm-long filling remnants promoted a poorer seal than that of an intact obturation.[12,15,29]

In the present study, the option to leave a 4-mm filling remnant was based on both the sealing capability and the interference of the barrier thickness on the depth of the intraradicular post.

Another aspect to be considered is the moment in which the post space preparation is performed. When such a space is prepared in the same session, at the end of the endodontic treatment - the so-called "immediate technique" - a factor to be considered is the absolute isolation of the tooth. This is a common situation during endodontic therapy, but an unusual one in interventions for post space preparation in later sessions, which may lead to infiltration of microorganisms into the RCS and possibly result in failure. Several studies support that post space preparations performed at a later date eventually result in greater infiltration, compared to those prepared immediately following root canal obturation,[30,32] a finding which supported our decision to adopt the immediate technique. However, Abramovitz et al. (2000) found no difference in the quality of the apical seal after immediate versus delayed post space preparation.[29]

Studies comparing the sealing capability of Cavit with other materials - such as resins, glass ionomers, IRM, and Super EBA - showed that the former performed better in most of the cases.[5,33-36] This better sealing capability can be attributed to its linear expansion up to pre-manipulation taking place during its setting time, and thus reducing handling-related inconsistencies. Moreover, Cavit contains no eugenol in its formula, and self-polymerises in the presence of humidity, from the reaction between water and calcium sulphate and zinc sulphate, both contained in its composition. It also promotes excellent sealing.[21,35,37-39] Cavit is not only easily inserted into the root canal, but also offers excellent working time, and is easily removed if retreatment or prosthetic intervention is needed. These properties were taken into account when this material was chosen as an intracanal barrier for the present study.

Most of the studies on material thickness used a 3-mm-thick sealant, on average, at the canal entrance.[21,35,37-39] Coltosol barriers 1-mm thick placed over 4-mm filling remnants were found to decrease infiltration in dog teeth, thus interfering positively with periapical healing.[16] Balto et al.[34] tested Cavit, IRM, and Temp Bond materials against infiltration of *E. faecalis*, but a cotton pellet was placed over the filling remnant instead of these materials, leaving a 3-mm space for later placement of these barriers. None of the three types of barriers were able to prevent coronal infiltration for a long period of time. Although the 30-day observation period of the Balto et al.[34] study was half of the experimental time adopted in the present study, the results were similar. This emphasizes how important it is to restore endodontically treated teeth prepared to receive an intraradicular post, as soon as possible.

Dual chamber models are generally made with marker agents that penetrate into the root canal fillings to assess the quality of sealant materials used as barriers against coronal infiltration.[5,25] We chose *Enterococcus faecalis*, a gram-positive facultative anaerobic microorganism, to serve as an infiltration marker, because this species is frequently related to persistent infections, and can be detected in most cases

of unsuccessful endodontic treatment. In addition, it can survive stressful environmental conditions such as those of nutrient scarcity.[40-42] This microorganism carried by contaminated food can even infiltrate restorative materials to reach the root canal system.[43]

In the present study, *E. faecalis* infiltration was found in all experimental groups, but 1- and 2-mm-thick barriers were able to delay infiltration by 18 and 26 days, respectively, in 90% of the specimens. Based on these findings, one can deduce that such a delay in infiltration enables the intraradicular post to be fitted and a definitive restoration to be placed in a clinical setting, before contamination of the root canal space occurs. Therefore, it is timely to recommend not only that the post should be fitted, but also that definitive restoration should also be placed as quickly as possible.

Conclusions

According to the parameters used in the present study, the following conclusions can be drawn:

1. Cavit barriers 1- and 2-mm thick were not able to prevent infiltration of *E. faecalis* during the entire experimental period, but did contribute positively to delaying the infiltration.

2. The experimental group receiving no type of barrier showed poorer protective performance, compared with both groups using specimens having 1- and 2-mm-thick Cavit barriers.

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