



Ex vivo analysis of marginal apical sealing ability of a MTA Fillapex[®]

Nilce Mendes Faria Pinto^a, Maria Eugênia Alvarez-Leite^a, Eduardo Nunes^a, Martinho Campolina Rebello Horta^a, Héctor Michel Rodrigues^a, Frank Ferreira Silveira^a

Abstract

Objective: The present *ex vivo* study aimed to assess the apical sealing ability of the endodontic sealer MTA FILLAPEX[®] compared to Sealapex, *Pulp Canal Sealer*, and AH26.

Methods: The ability to prevent leakage of a culture of *Enterococcus faecalis* through the root canal obturation was assessed. Forty-eight single-rooted teeth that had been extracted from humans were used. Following instrumentation and obturation using the warm gutta-percha vertical condensation technique, the teeth were allocated into one of four experimental groups (n=10), a positive control group (n=4), or a negative control group (n=4). The microbial inoculation was performed every three days over 60 days. The data were tabulated and subjected to statistical survival analysis, whereby the performance of the four investigated sealers was compared using the log-rank test.

Results: The results revealed that all of the samples in the group in which MTA FILLAPEX[®] was used exhibited leakage. Sealapex and Pulp Canal Sealer exhibited leakage in 90 and 80% of the samples, respectively. The sealer AH26 was significantly superior in delaying leakage, although 30% of the samples exhibited leakage.

Conclusion: MTA FILLAPEX[®] permitted the most *Enterococcus faecalis* leakage compared to the other investigated sealers.

Key words: Dental cements; Root canal obturation; Root canal therapy

^a Departamento de Odontologia – Pontifícia Universidade Católica de Minas, Belo Horizonte, Minas Gerais, Brasil.

Avaliação *ex vivo* do selamento apical marginal do MTA FILLAPEX[®]

Resumo

Objetivos: O presente estudo teve como objetivo avaliar *ex vivo* a capacidade de selamento apical do cimento endodôntico MTA FILLAPEX[®] comparando com Sealapex, *Pulp Canal Sealer* e AH26.

Metodologia: Foi avaliada a capacidade deste cimento em prevenir a infiltração de uma cultura de *Enterococcus Faecalis* através da obturação do canal radicular. Foram utilizados 48 dentes unirradiculados de humanos, que depois de instrumentados e obturados pela técnica da condensação vertical da guta-percha aquecida, foram divididos em 4 grupos experimentais (n=10), um grupo controle positivo (n=4), ou um grupo controle negativo (n=4). A inoculação microbiana foi realizada a cada três dias, sempre com cultura de 24 horas, durante um período de 60 dias. Os dados foram tabulados e analisados estatisticamente por Análise de Sobrevida, onde se comparou o desempenho dos quatro cimentos, utilizando o Teste Log-Rank.

Resultados: Os resultados mostraram que o grupo obturado com MTA FILLAPEX[®], apresentou todas as amostras infiltradas. Os cimentos Sealapex e Pulp Canal Sealer apresentaram 90 e 80 e 30% de amostras infiltradas respectivamente. O cimento AH26 foi significativamente mais eficiente em retardar a infiltração, embora com 30% das amostras infiltradas.

Conclusão: O cimento MTA FILLAPEX[®] quando comparado aos outros cimentos avaliados, apresentou a maior infiltração frente a uma cultura de *Enterococcus Faecalis*.

Palavras-chave: Cimentos endodônticos; Obturação do canal radicular; tratamento do canal radicular

Correspondence:
Frank Ferreira Silveira
frankfouii@uol.com.br

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Introduction

In all areas of dentistry, explanations for observations that are not fully understood are tirelessly sought to improve knowledge, techniques, and the materials that are used in clinical practice. The important roles that microorganisms and their by-products play in the pathogenesis of pulpal and periapical diseases are well established [1]. When strictly anaerobic techniques are applied to endodontic cultures, many infections that were formerly caused by aerobic or facultative bacteria are now classified as polymicrobial infections, where anaerobic bacteria are prevalent. The primary aim of endodontic therapy is to prevent and treat periradicular inflammation by eliminating microorganisms from the root canal system (RCS). The methods that are commonly used for this purpose include antimicrobial irrigation, cleansing, and appropriate obturation of the RCS, followed by coronal restoration. Modern endodontic therapy follows basic principles that are grounded on well-structured scientific foundations that guide all of the stages of treatment. Although the success of endodontic treatment depends on the attention that is given during all stages, i.e., from when the medical history is obtained through follow-up, three-dimensional hermetic obturation of the RCS is a crucial step [2]. Such obturation prevents the percolation and microleakage of the periapical exudate into the internal canal space, hindering reinfection. Moreover, obturation promotes an environment that is biologically favorable to the tissue healing.

Two features must be taken into account in the study of sealers, namely, physicochemical factors and biocompatibility. Moreover, antimicrobial activity may play an important role in the efficacy of endodontic sealers in the obturation of root canals. However, no endodontic sealer exhibits all of the desirable properties, but each has certain advantages.

An ideal endodontic sealer must exhibit the following properties: easy insertion and removal from the root canal; satisfactory working time; promotion of three-dimensional sealing of the RCS; dimensional stability under conditions of use; satisfactory drainage; radiopacity; non-staining of the dental structures; satisfactory adhesion to the canal walls; cohesive strength; insolubility in tissue fluids and saliva; solubility or resorbability in the periradicular tissues; impermeability in the canals; biocompatibility; and antimicrobial activity.

Recently, a new MTA-derived endodontic sealer was released, under the name MTA FILLAPEX®. Mineral trioxide aggregate (MTA) is a powder that is composed by fine hydrophilic particles of tricalcium silicate, dicalcium silicate, tricalcium aluminum, calcium sulfate, and bismuth oxide. MTA is known for its biocompatibility, effective sealing properties, low solubility, and a radiopacity that is slightly greater than that of dentin [3]. MTA was initially used to treat root perforations, and later in apical sealing due to its ability to solidify under humid conditions [4,5].

There are few data regarding the sealing ability of MTA FILLAPEX®; it therefore appears appropriate to assess its sealing properties and to compare it to other endodontic sealers that are widely mentioned in the international literature.

Methods

The present study was approved by the Research Ethics Committee of the Pontifical Catholic University of Minas Gerais (CAAE – 0292.0.213.000-10).

Sample selection

A total of 48 single-rooted human teeth were supplied by the PUC Minas tooth bank for this study. The inclusion criteria were the following: teeth exhibiting straight canals and roots, complete rhizogenesis, and a minimum of a 13 mm between the cervical margin and the root apex.

Sample preparation

Following sterilization in an autoclave, the crowns were sectioned at the level of the cement-enamel junction using a carborundum disk that was mounted in a mandrel and activated by a straight micro motor handpiece. The roots were then stored in distilled water and 2.5% sodium hypochlorite at a 10:1 ratio until the preparation of the RCS. After the access cavity was prepared, canal patency was obtained by inserting a #15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) inside of the canal until it was observed through the apical foramen. The canal patency length (PL) was defined as the measurement between the instrument tip and the cervical reference of the root. The working length (WL) was obtained by subtracting one mm from the PL. The cleansing and shaping of the RCS were performed using a *ProTaper* rotary instrumentation system (Dentsply Maillefer, Ballaigues, Switzerland) in combination with hand instrumentation (K-files). To improve the standardization and the removal of anatomical interferences from the foramen area, the foramina of all of the specimens were cleansed by the sequential use of #15, 20, 25, and 30 K-files. Abundant irrigation with 2 ml of 2.5% sodium hypochlorite solution was applied at every change of instruments using a 5-ml disposable syringe (Injex, Ourinhos, Brazil) and a 25-gauge cannula (Injex, Ourinhos, Brazil). The canal patency was maintained throughout the cleansing and shaping procedures using a #30 K-file. After the instrumentation was completed, the canal was irrigated with 3 ml of 17% EDTA for 3 minutes, followed by irrigation with 2 ml of 2.5% sodium hypochlorite; the canal was then dried with #30 absorbent paper points (Dentsply Maillefer, Ballaigues, Switzerland). The obturation of the RCS was performed using the vertical condensation technique with thermoplastic gutta-percha. Size M master cones were used (Dentsply Maillefer, Ballaigues, Switzerland), which were adjusted to 1 mm beyond the PL using a calibration ruler (Dentsply Maillefer, Ballaigues, Switzerland) that was

adjusted to a #35 diameter. The adaptation of the cone was verified using conventional periapical radiography. For the obturation of the root canals, the teeth were allocated into four experimental groups depending on the sealer that was used, as described in Table 1.

Table 1. Distribution of groups

Groups	Sealer	n
1	MTAFillapex	10
2I	Sealapex	10
3	Pulp Canal Sealer	10
4	AH 26	10

During the obturation, the gradual removal of the gutta-percha towards the apex was performed using fine-medium condenser tips that were coupled to a *System B* device (Analytic Technology, Redmond, WA, USA). Vertical compaction was performed using #2 Schilder condenser (Odous, Belo Horizonte, Brazil). Next, the canal was filled via the successive addition of small amounts of gutta-percha. Radiographs were taken to assess the quality of the obturation. The obturation in the negative and positive control groups was performed using only one gutta-percha M accessory cone, which was adjusted to diameter #35 without the use of an endodontic sealer.

Preparation of the testing apparatus

The testing apparatus that was used to examine the dual-chamber experimental model consisted of a structure that included 10-ml glass vials (Wheaton do Brasil S.A., São Bernardo do Campo, Brazil), 20-mm diameter rubber caps (Adnaly Artefatos de Borracha Ltda, São Paulo, Brazil), and 1.5-ml Eppendorf tubes (Cral, Comércio de Artigos para Laboratório, São Paulo, Brazil). Holes with 11 mm of diameter were made at the center of the rubber caps using a steel perforator (Indústria e Comércio Graziano, São Paulo, Brazil), and the ends of the Eppendorf tubes were shortened by 7 mm using a carborundum disk that was mounted in a mandrel and activated using a straight micro motor handpiece.

The teeth were placed in the Eppendorf tubes after the flaming of their cut ends to achieve improved adaptation and adjustment of the cervical third. The specimens in groups 01, 02, 03, and 04, and the positive control were sealed, except for the 3 apical mm, with two layers of cyanoacrylate (Super Bonder, Henkel Loctite Adesivos Ltda., Itapevi, Brazil). The sealant was applied using a brush, and a 1-hour interval was waited between the applications. The radicular structure of the negative control group was fully sealed, including the 3 apical mm and the entire middle and cervical thirds of the roots, using the same sealing agents.

The specimens were kept at room temperature until the drying was complete. Next, a layer of nail polish (Colorama

Cremoso, Procosa Produtos de Beleza Ltda, São Paulo, Brazil) was applied.

After drying, the Eppendorf tube-tooth portion was sealed with one layer of epoxy resin (Durepóxi, Henkel Loctite Adesivos Ltda, Itapevi, Brazil).

Next, one layer of cyanoacrylate was applied on both the epoxy resin and the sealed root surfaces, followed by one additional layer of nail polish.

The sealing agents were left to dry at room temperature for 24 hours. Next, all of the testing devices, including the Eppendorf tubes-teeth, the 10-ml glass vials, and the perforated rubber caps, were duly identified, and the sets were individually numbered prior to sterilization in ethylene oxide gas (Curar Centro de Esterilização Ltda, Belo Horizonte, Brazil). The sterilization was performed by means of exposure to the gas for 240 minutes at 55 °C and 60% relative humidity. This step was followed by aeration for 180 minutes.

Following the performance of a protocol that was modified from that described in Valadares et al. [6], a microbial marker from the *American Type Culture Collection* was used (*Enterococcus faecalis* – ATCC 4083).

Brain Heart Infusion (BHI) broth culture medium (BHI Difco Laboratories, Detroit, MI, USA) was prepared according to the manufacturer's instructions and sterilized in an autoclave. The flask that contained the sterilized culture medium, together with the individual wraps of the testing devices that were sterilized in ethylene oxide gas, were opened under a laminar flow hood, where the fixation platform was assembled. The culture medium was distributed among the glass vials.

A total of 6.5 ml of BHI broth were placed in each vial, which were then covered with the perforated caps. Lastly, the Eppendorf tube-tooth sets were immersed in the culture medium to a depth that corresponded to approximately 3 mm of the roots.

The strain was propagated using weekly inoculations in Petri dishes that contained BHI agar (BHI Difco Laboratories, Detroit, MI, USA). A bacterial suspension was prepared from a 24-hour culture in BHI agar in 5 ml of sterile distilled water with a turbidity that corresponded to the McFarland standard 1.0 (3×10^8 cells/ml).

A 1-ml aliquot of that suspension was used to prepare a new suspension with 5 ml of BHI broth, from which 0.1 ml was used to inoculate the specimens at the upper chamber of the experimental model, i.e., in the Eppendorf tube-tooth sets. These samples were then incubated in a bacteriological oven at 37 °C under aerobic conditions. Microbial inoculation was performed every three days, always with 24-hour cultures, over a period of 60 days.

The microbial viability and load were tested at each inoculation by inoculating 0.1 ml of microbial suspension into a test tube that contained 10 ml of BHI broth.

The presence or absence of turbidity in the culture medium in the glass vials was assessed daily throughout the study. This measure served as an index of the presence or absence of microorganisms, which in turn characterized microbial

leakage through the RCS. These observations were entered in spreadsheets according to the experimental group.

Statistical analysis

The data were arranged in survival curves, and the difference between the groups was analyzed using the log-rank test (Mantel-Cox). The significance level was set at 5%. The analysis was performed using GraphPad Prism 5.00 software (GraphPad Software, San Diego, CA, USA).

Results

All of the specimens in the positive control group exhibited leakage within 24 hours, whereas the culture medium in the negative control group did not exhibit turbidity over the 60 days of the study.

With respect to the percentage of samples with leakage, 100% of the positive control group samples, 100% of the samples in experimental group 1, 80% of the samples in experimental group 2, 90% of the samples in experimental group 3, and 30% of the samples in experimental group 4 exhibited leakage.

The log-rank test revealed difference between the groups ($p < 0.05$). This test, in combination with the assessment of the survival curves allow us to conclude that leakage was delayed in G4 compared to the other groups.

Figure 1 depicts the survival curves of the four investigated groups and the percentage of non-leaking samples.

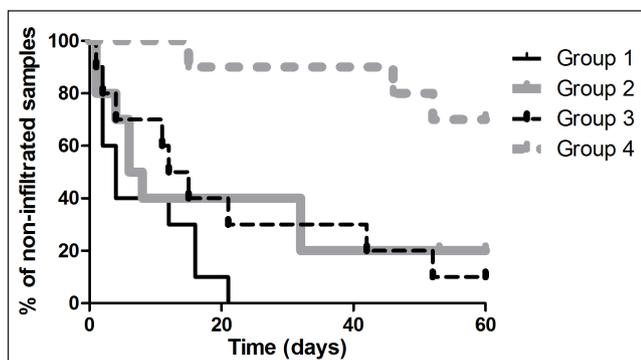


Figure 1. Survival curves (leakage) in days.

Discussion

Modern RCS obturation techniques prioritize a larger gutta-percha amount and less sealer film given that the latter is the weak portion of the obturation. Nevertheless, endodontic sealers play an important role in the control of apical percolation in that they flow into the branches and improve the adaptation of the obturation to the irregularities that are exhibited by the dentin-filling material interface [7].

Certain studies have assessed apical sealing following obturation of root canals using different sealers, including dye leakage, fluid passage, and biological markers [8,9]. In

the present study, a method that investigates apical leakage using microbial markers was selected because (i) well-defined results can be obtained and (ii) more importantly, this method best matches the conditions that are met in actual clinical practice.

The majority of the experimental models that are used to examine micro-leakage that employ biological markers (i.e., microorganisms or saliva) use a dual-chamber system. In this system, the microbial marker is inoculated in combination with a culture medium in the upper chamber, and contamination is assessed in the lower chamber [6,10-17].

In 1996, Chailertvanitkul et al. [18] investigated the coronal leakage of obligate anaerobes using *Fusobacterium nucleatum*. In 1997, Chailertvanitkul et al. [11] investigated microbial leakage in obturated canals by storing the specimens in artificial saliva and using *Streptococcus sanguinis* and *Prevotella intermedia* to test for leakage. The present study used *Enterococcus faecalis* because it is the microorganism that is more frequently associated with endodontic failure and with the persistence of periapical lesions [19,20]. Moreover, other authors have used *Enterococcus faecalis* in studies that have tested endodontic sealers [21,22].

In the present study, the performance of sealer MTA FILLAPEX® to prevent bacterial leakage was unsatisfactory. As this sealer was recently released, it was not possible to compare the results of the present study to those of other studies.

In agreement with the findings by Almeida et al. [23], Sealapex exhibited less leakage compared to Pulp Canal Sealer. In this previous study, AH Plus, Epiphany, and Sealapex exhibited improved sealing ability compared to Pulp Canal Sealer. Thus, the superior performance of the resin sealer AH26 in the present study is consistent with several previous studies [24,25].

The desirable properties of endodontic sealers have been described, but most sealers exhibit different limitations. Therefore, further studies must be performed to minimize their adverse effects and to provide data to professionals that will allow them to achieve success in dental clinical practice.

Conclusions

Based on the present results, and considering the specific circumstances of the present study, one may conclude that: (i) no sealer was efficient in avoiding leakage; (ii) the AH26 sealer exhibited the greatest ability to delay *Enterococcus faecalis* leakage; (iii) the MTA FILLAPEX® sealer exhibited the greatest *Enterococcus faecalis* leakage compared with the other investigated sealers.

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