Pesquisa Brasileira em Odontopediatria e Clínica Integrada

ISSN: 1519-0501

apesb@terra.com.br

Universidade Federal da Paraíba

Brasil

Rodrigues, Hector Michel; Nunes, Eduardo; Alvarez - Leite, Maria Eugênia; Campolina Horta, Martinho; Faria Pinto, Nilce; Ferreira Silveira, Frank

Evaluation of Bacterial Infiltration in Teeth with Apical Barrier of MTA: An ex-vivo Study

Pesquisa Brasileira em Odontopediatria e Clínica Integrada, vol. 14, núm. 4, 2014

Universidade Federal da Paraíba

Paraíba, Brasil

Brazilian Research in Pediatric Dentistry and Integrated Clinic 2014, 14(4):325-333
DOI: http://dx.doi.org/10.4034/PBOCI.2014.144.07
ISSN 1519-0501

Original Article

Evaluation of Bacterial Infiltration in Teeth with Apical Barrier of MTA: An ex-vivo Study

Hector Michel Rodrigues[1], Eduardo Nunes[2], Maria Eugênia Alvarez-Leite[2], Martinho Campolina Horta[2], Nilce Faria Pinto[2], Frank Ferreira Silveira[2]

[1]Dentist, Belo Horizonte, MG, Brazil.
[2]Department of Dentistry, Pontificial Catholic University of Minas Gerais (PUC), Belo Horizonte, MG, Brazil.

Author to whom correspondence should be addressed: Frank Ferreira Silveira, Avenida Dom José Gaspar, 500, Prédio 46, Coração Eucarístico, Belo Horizonte, MG, 30535-610, Brasil. Phone: 55 (31) 33194414. E-mail: frankfoui@uol.com.br.

Academic Editors: Alessandro Leite Cavalcanti and Wilton Wilney Nascimento Padilha

Received: 07 February 2014 / Accepted: 10 September 2014 / Published: 12 December 2014

Abstract

Objective: To evaluate the ability of different MTA thicknesses (3 and 5 mm), with or without conventional fillings, in preventing bacterial infiltration of Enterococcus faecalis in teeth with incompletely formed apices. **Material and Methods:** Apical barriers were created which 3- and 5-mm-thick of MTA with and without conventional filling of the remaining root. 48 single-rooted human teeth were divided into four experimental groups (n=10) and two controls groups (n=8). After being mounted on a testing apparatus, Enterococcus faecalis was used as an indicator of bacterial infiltration. Inoculations were renewed every 3 days during a period of 60 days, and infiltration was assessed daily by checking the culture medium for turbidity. The results were analyzed using descriptive and analytical statistics (survival analysis). The level of significance was set at 5%. The tests were performed using the software GraphPad Prism 5.00. **Results:** The positive control group infiltrated within 24 hours, while the negative control group, no infiltrated. The Long-rank test did not demonstrate the existence of differences between groups. However, the Long-rank test for tendencies showed of a tendency to retard the infiltration. **Conclusion:** None of the MTA barriers were effective in preventing bacterial infiltration. The gutta-percha filling of the root segment in association with the 5-mm apical barrier of MTA showed a tendency to delay bacterial infiltration.

Keywords: Endodontics; Enterococcus faecalis; Root canal therapy.

.

Introduction

Apexification is a method for inducing apical closure in a non-vital tooth with incomplete root formation through the establishment of a mineralized barrier. This procedure is typically performed with calcium hydroxide paste, requiring multiple visits to the dental clinic for successive exchanges, and the complete formation of the apical barrier can be prolonged depending on the stage of root development [1].

Endodontic treatment in teeth with open apices or large foramen requires special care from the professional, differing from conventional treatment due to this anatomic particularity. This apical anatomy is characterized by a greater width of the apical portion compared with the cervical portion, in addition to the absence of apical constriction and difficulty for the corrected determination of the working length. The need for multiple sessions increases the likelihood of microorganism (MO) leakage or even the risk of tooth fracture [2-4].

Mineral Trioxide Aggregate (MTA) was developed at the Loma Linda University (USA) and was initially advocated to seal the communication routes between root canal system (RCS) and surrounding tissues [5]. In addition to biocompatibility, MTA is characterized by dimensional stability, insolubility in tissue fluids and good behavior in the presence of humidity. The tissue response induced by contact with MTA is characterized by the neoformation of mineralized tissue [6]. However, technical difficulties in applying the material and in controlling its extrusion may interfere with its adoption [7].

The use of MTA on the apical region can facilitate a faster implementation of the obturation, enabling faster tooth restoration and possibly resulting in a more favorable long-term prognosis [8]. This conduct promotes a reduction in the duration and cost of treatment, better patient cooperation and the possible elimination of problems that may arise during extensive treatments, such as difficulty in maintaining adequate temporary sealing and the integrity of the remaining tooth [9,10].

A classic study [11] has established the role of bacteria in the etiology and maintenance of pulpal and periapical pathologies. Therefore, endodontic therapy should be performed to eliminate bacteria and prevent reinfection of the RCS. Endodontic infections are caused by MOs that have access to the pulp and periapical tissues, presenting a typically polymicrobial nature, with a predominance of anaerobic bacteria. Enterococcus faecalis is a bacterium that is associated with most endodontic treatment failures and is recognized for its great resistance to drug therapies [12].

The present study aimed to evaluate the ability of different MTA thicknesses (3 and 5 mm), with or without conventional fillings, in preventing bacterial infiltration of Enterococcus faecalis in teeth with incompletely formed apices.

Material and Methods

A total of 48 single-rooted permanent human teeth, originating from the tooth bank at the Pontifical Catholic University of Minas Gerais, were used. Following radiographic evaluation, the criteria for inclusion in the study were as follows: straight roots and root canals with complete root

formation and 13 mm measured from the cervical margin to the apex of the root. The crowns and 3 mm of the apices were removed using a carborundum disk; thus, all of the roots had a standard length of 10 mm.

Once the entrance to the root canal was located, a # 50 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was introduced into the canal until it could be observed at the opening of the apical foramen, and this measurement was used to determine the working length. The instrumentation of the canal was performed with # 6 to 1 Gates Glidden drills (Dentsply Maillefer, Ballaigues, Switzerland) progressively inserted in the crown-apex direction, of which drill # 1 was used beyond the apical foramen [13]. The divergence of the apical preparation was performed, reaching a diameter of 1.36 mm in the foramen through retrograde instrumentation with a # 40 Profile file of 0.06 conicity, inserted to the full length of its active portion [13]. After each step of the surgical procedure, the teeth were irrigated with 1 mL of 5.25% NaOCl (Lenza Farmacêutica, Belo Horizonte, Brazil). The canals were dried with # 80 absorbent paper tips (Tanari, Tanariman Ind. Ltda., Manacapuru, Brazil), and the integrity and shape of the apical foramina were checked using a surgical microscope (Alliance do Brazil, São Paulo, Brazil).

The teeth were fixed in a vise, and the apical region was supported by a moist sponge to provide a slight resistance to material extrusion at the moment of filling. The proportion and manipulation were performed according to the manufacturer's recommendations and accommodated as close as possible to the apex using an MTA carrier (Angelus, Londrina, Brazil), with the help of the surgical microscope. The white MTA (Angelus, Londrina, Brazil) was applied using gentle pressure with # 4, 5 and 6 Schilder pluggers (Odous De Deus, Belo Horizonte, Brazil), using a penetration limiter, until the desired thickness was achieved. The teeth were radiographed to determine the level of apical filling. Number 80 paper tips moistened with saline solution (Tanari, Tanariman Ind. Ltda., Manacapuru, Brazil) were introduced into the root canal for 30 minutes to accelerate cement setting. In the groups with gutta-percha filling, Pulp Canal Sealer cement was previously brushed onto the canal walls with a # 70 file, followed by the Obtura II thermoplasticized gutta-percha injection (Spartan, Fenton, USA).

Table 1. Experimental groups.

	MTA Barrier	Obturation
GI (n=10)	3 mm	Absent
GII (n=10)	3 mm	Present
GIII (n=10)	5 mm	Absent
GIV (n=10)	5 mm	Present

One positive control group (n=1) (root with no impermeability and no MTA barrier) and one negative control (n=1) (completely impermeable root) were used for each experimental group.

Following the methodology previously described [14], a dual-chamber experimental model was made for the construction of the test apparatus, using 10.0-mL glass vials (Wheaton do Brasil

S.A., São Bernardo do Campo, Brazil) with rubber stoppers 20.0 mm in diameter (Adnaloy Artefatos de Borracha Ltda,São Paulo, Brazil) and 1.5-mL Eppendorf tubes (Cral, Comércio de Artigos para Laboratório, São Paulo, Brazil). The caps were perforated in the center with a steel drill (Ind. Com. Graziano Ltda., São Paulo, Brazil) to establish an 11 mm-diameter opening. In the Eppendorf tubes, 7 mm of the tips were sectioned, after which the tips were heated, and the specimens were inserted under pressure for a better fit.

To seal the samples, two layers of cyanoacrylate (Super Bonder, Henkel Loctite Adesivos Ltda., Itapevi, Brazil) were used, in addition to a layer of nail polish (Colorama Cremoso, Procosa Produtos de Beleza, São Paulo, Brazil), except on the 3 mm of the apex. The Eppendorf tube-tooth junction was subsequently sealed with epoxy resin (Durepóxi, Alba Química, Boituva, Brazil),followed by another layer of cyanoacrylate and a layer of nail polish. Twenty-four hours later, the test apparatus was sterilized with ethylene oxide gas (Curar Centro de Esterilização, Belo Horizonte, Brazil). In a laminar flow chamber, each glass vial received 6.5 mL of Brain Heart Infusion (BHI) medium (Difco Laboratories, Detroit, USA), and the Eppendorf tube-tooth set was subsequently inserted into the stopper until 3 mm of the apex was immersed into the medium. A fresh culture of E. faecalis ATCC 4083 was inoculated in 5.0 mL of sterile distilled water until a turbidity equivalent to McFarland # 1 was achieved, which represented approximately 3 x 108 cells/mL. A 1.0-mL aliquot was removed from this microbial content for the preparation of a new suspension in 5.0 mL of BHI broth, from which 1 mL was removed, in turn, for the inoculation of the specimens in the upper chamber of the test apparatus. This set was incubated under aerobic conditions in a bacteriological oven at 37°C, and new inoculations of fresh microbial suspension were performed every 3 days for 60 days. Daily assessment for the presence or absence of turbidity was performed because turbidity presence indicated the passage of microorganisms through the root canal system. For each of the 10 teeth evaluated in each group was recorded the day that this presence of bacterial infiltration or absence of bacterial leakage occurred at the end of the experiment.

To determine the viability of E. faecalis, 0.1 mL of the inoculating suspension and 0.1 mL of the content already inoculated in the Eppendorf tube-tooth were removed at each inoculation, placed into tubes containing 10.0 mL of BHI broth and kept in a bacteriological oven.

To determine whether the contamination originated from the same biological marker used for inoculation, smears were made for all samples with turbid culture medium using the Gram method.

The results were analyzed using descriptive statistics (median, minimum and maximum values) and analytical statistics (survival analysis). In survival analysis, the results of each group were arranged in survival curves indicating the time (in days) elapsed until the next event: the presence of bacterial infiltration. Differences between groups were analyzed using the log-rank (Mantel-Cox) test. The existence of trend between the groups was analyzed by the log-rank test for

trend. The level of significance was set at 5%. The tests were performed using the software GraphPad Prism 5.00 (GraphPad Software, San Diego, CA, USA).

Results

The log-rank test showed no difference in survival between the groups ($p > 0.05$). However, the log-rank test for trend demonstrated the existence of trends in survival between the groups ($p < 0.05$). The result of this last test, linked to the observation of the survival curves (Graphic 1) and median survival (Chart 1), allows us to indicate a trend toward greater survival in Group 4, ie, a tendency to delayed bacterial infiltration group 4.

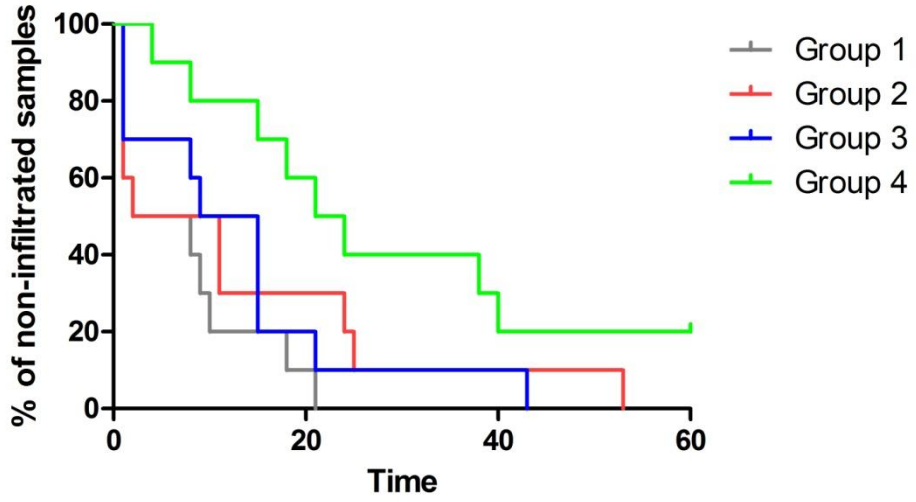

Figure 1: Survival curves indicating the time (in days) elapsed until the next event: the presence of bacterial infiltration.

Table 2. Median, minimum and maximum survival (time until the occurrence of bacterial infiltration) in the 4 groups.

Group 1	Group 2	Group 3	Group 4
5 (1 – 21)	6,5 (1 – 53)	12 (1 – 43)	22,5 (1 – [a])

[a]Group 4 there was no bacterial infiltration in two teeth, even the maximum elapsed time of the experiment (60 days).

Discussion

Calcium hydroxide is traditionally used to induce biological sealing in teeth with incompletely formed apices [1,2]. Its main disadvantages are the unpredictable and prolonged course necessary for treatment completion, requiring multiple visits to the clinic, which demands high patient commitment [10]. Furthermore, this technique presents risks of reinfection through temporary sealing material [15] and the possibility of tooth fracture [3,4].

MTA, initially recommended as a retro-obturator material [16], is an excellent alternative for inducing biological sealing in cases of apexification [6]. Moreover, this compound enables faster treatment completion, providing a high rate of clinical success [8,17].

Another methodology, the infiltration of a protein-dye complex and infiltration of dye has been used for the in vitro assessment of the efficacy of MTA barriers. The infiltration of MOs (E. faecalis) was assessed in the present study, similar to that described in other studies [20,21].

Root standardization was achieved by removing the crown and 3 mm of the apex, with the purpose of centralizing the foramen and eliminating possible apical deltas, as advocated by several authors [9,20].

The simulation of teeth with incompletely formed apices was performed according other studies [9,13], who used # 6 to 1 Gates Glidden drills in the crown-apex direction, with the # 1 drill used to surpass the foramen. The divergence of the apical preparation was performed with a retrograde instrumentation of the root canal using a # 40 Profile file of 0.06 conicity inserted to the full length of its active portion [13].

Regarding the apical barrier of MTA, the literature shows that several thicknesses have been employed. One study [19] have tested 1-mm-thick barriers, although most of the studies and clinical case reports have used 3- to 5-mm-thick barriers [9,17-19,21]. In the present study, the authors used 3- and 5-mm barriers of MTA, taking into account that the remaining root would be filled with conventional filling material in two groups.

None of the barriers used was capable of preventing E. faecalis infiltration. In teeth filled with 5 mm of MTA, a tendency toward delayed infiltration was noted compared with teeth filled with 3 mm only, which is consistent with results from other studies [18,20,22]. When completing the root canal filling with conventional obturation material, a slower progression of leakage was noted in the teeth with 5-mm barriers of MTA. This finding is clinically relevant, given that the completion of the final restoration of the tooth is often delayed, which can compromise the effectiveness of RCS sealing.

The orthograde insertion of MTA is considered to be a sensitive technique because, in addition to the difficulty in transporting the material to the region, condensation is limited due to the lack of resistance in the apical region. Several methods have been recommended, and their efficacy is influenced by the modus operandi. In the present study, the procedure was performed with the aid of a surgical microscope. Notably, the use of a surgical microscope in the clinical procedure makes it easier to control the insertion of the material into the apical region, minimizing its extrusion, as observed in several studies [22-24].

As used in other studies, the use of a specific MTA carrier and hand plugger was the method chosen for the present study [20,22,24]. An alternative method would be the concomitant use of appropriate tips attached to the ultrasound device, vibrating directly on the plugger [21,24-29].

The irregularities and divergent nature of the apical anatomy can limit the adaptation to the dentin walls, creating gaps in the marginal interface of the dentin. Therefore, despite the limitations

of conventional periapical radiographic evaluation, this procedure was used to assess the adaptation of the MTA, a procedure that has also been recommended by other authors [18,22,23].

It is worth noting the rationale for the choice of the statistical method employed in this study. The survival analyzes are used to evaluate a phenomenon in relation to the time elapsed until the occurrence of a particular event final. Therefore, this statistical technique not only measures the final event "death" (as suggested by the term "survival"), but also other events with dichotomous outcomes, such as the recurrence of a disease [30]. In the present study, the outcome were the occurrence of bacterial infiltration.

Several benefits have been associated with the use of MTA as an apical barrier in teeth with incomplete root formation, particularly in relation to the reduction of time taken for treatment completion, which minimizes the potential risk of tooth fracture. However, thorough application is essential to prevent unwanted leakage of material, which can lead to failure of the treatment [31]. Notably, the present study method simulates, in vitro, a situation that is very similar to the clinical conditions encountered in the periradicular region, although it should be pointed out its limitations. Further studies should be encouraged to definitely determine the clinical use of MTA in the treatment of teeth with incomplete root formation. However, for conducting clinical research, ethical issues and compromised patient may increase the difficulty of this challenge.

Conclusions

None of the MTA barriers were effective in preventing bacterial infiltration. The gutta-percha filling of the root segment in association with the 5-mm apical barrier of MTA showed a tendency to delay bacterial infiltration.

Acknowledgements

This study was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq, Fundação de Amparo à Pesquisa do Estado de Minas Gerais - FAPEMIG, and Fundo de Incentivo à Pesquisa da PUC Minas (FIP) - PUC Minas, Brazil.

References

1. Morse DR, O'Larnic J, Yesilsoy C. Apexification: review of the literature. Quintessence Int 1990; 7(21):589-98.

2. Heithersay GS. Calcium hydroxide in the treatment of pulpless with associated pathology. J Br Endod Soc 1975; 8(2):74-91.

3. Ricketts DN, Tait CM, Higgins AJ. Restauration of the root-filled tooth: pre operative assessment. Br Dent J 2005; 198(8):463-71.

4. Al Ansary MAD, Day PF, Duggal MS, Brunton PA. Interventions for treating traumatized necrotic imature permanent anterior teeth: inducing a calcific barrier & root strengthening. Dent Traumatol 2009; 25(4):367-79.

5. Lee SJ, Monsef M, Torabinejad M. The sealing ability of a mineral trioxide aggregate for repair of lateral root perforations. J Endod 1993; 19(11):541-4.

6. Pitt Ford TR, Torabinejad M, McKendry DJ, Hong CU, Kariyawasam SP. Use of mineral trioxide aggregate for repair of furcal perforations. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1995; 79(6):756-63.
7. Tahan E, Celik D, Er K, Tasdemir T. Effect of unintentionally extruded mineral trióxide aggregate in treatment of tooth with periradicular lesion: A case report. J Endod 2010; 36(4):760-3.
8. Witherspoon DE, Small JC, Regan JD, Nunn M. Retrospective analysis of open apex teeth obturated with Mineral Trioxide Aggregate. J Endod 2008; 34(10):1171-6.
9. Martin RL, Monticelli F, Brackett WW, et al. Sealing properties of Mineral Trioxide Aggregate orthograde apical plugs and root fillings in an In Vitro model. J Endod 2007; 33(3):272-5.
10. Simon S, Rilliard F, Berdal A, Machtou P. The use of mineral trioxide aggregate in one-visit apexification treatment: a prospective study. Int Endod J 2007; 40(3):186-97.
11. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1965; 20(3):340-9.
12. Rôças IN, Siqueira Jr JF, Santos KRM. Association of Enterococcus faecalis with different forms of periradicular diseases. J Endod 2004; 30(5) 315-20.
13. De Deus G, Audi C, Murad C, Fidel S, Fidel R. Similar expression of through-and-through fluid movement along orthograde apical plugs of MTA BioTM and white Portland cement. Int Endod J 2008; 41(12):1047-53.
14. Valadares MA, Soares JA, Nogueira CC, Cortes MI, Leite ME, Nunes E, Silveira FF. The efficacy of a cervical barrier in preventing microleakage of Enterococcus faecalis in endodontically treated teeth. Gen Dent 2011; 59(1):32-7.
15. El-Meligy OAS, Avery DR. Comparison of apexification with mineral trioxide aggregate and calcium hydroxide. Pediatr Dent 2006; 28(3):248-53.
16. Torabinejad M, Rastegar AF, Kettering JD, Pitt Ford TR. Bacterial leakage of mineral trioxide aggregate as a root-end filling material. J Endod 1995; 21(3):109-12.
17. Torabinejad M, Chivian N. Clinical Applications of Mineral Trioxide Aggregate. J Endod 1999; 25(3):197-205.
18. Al-Kahtani A, Shostad S, Schifferle R, Bhambhani S. In vitro evaluation of microleakage of an orthograde apical plug of mineral trioxide aggregate in permanent teeth with simulated immature apices. J Endod 2005; 31(2):117-9.
19. Lolayekar N, Bhat SS, Hegde S. Sealing ability of ProRoot MTA and MTA Angelus simulating a one-step apical barrier technique an in vitro study. J Clin Pediat Dent 2009; 33(4):305-10.
20. Valois CRA, Costa Jr ED. Influence of the thickness of mineral trioxide aggregate on sealing ability of root-end fillings in vitro. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004; 97(1):108-11.
21. Kim US, Shin SJ, Chang SW, Yoo HM, Park DS. In vitro evaluation of bacterial leakage resistance of an ultrasonically placedmineral trioxide aggregate orthograde apical plug in teeth with wide open apexes: a preliminary study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009; 107(4):e-52-6.
22. Pace R, Giuliani V, Pini Prato L, Baccetti T, Pagavino G. Apical plug technique using mineral trioxide aggregate: results from a case series. Int Endod J 2007; 40(6):478-84.
23. Ghaziani P, Aghasizadeh N, Nezami MS. Endodontic treatment with MTA apical plugs: a case report. J Oral Sci 2007; 49(4):325-9.
24. Holden DT, Schwartz SA, Kirkpatrick TC, Schindler WG. Clinical outcomes of artificial root-end barriers with mineral trioxide aggregate in teeth with immature apices. J Endod 2008; 37(7):812-7.
25. Bogen G, Kuttler S. Mineral trioxide aggregate obturation: A review and case series. J Endod 2009; 35(6):777-90.
26. Aminoshariae A, Hartwell GR, Moon PC. Placement of mineral trioxide aggregate using two diferent techniques. J Endod 2003; 29(10):679-82.
27. Lawley GR, Schindler WG, Walker WA, Kolodrubetz D. Evaluation of ultrasonically placed MTA and fracture resistance with intracanal composite resin in a model of apexification. J Endod 2004; 30(3):167-72.
28. Yeung P, Liewehr FR, Moon PC. A Quantative Comparison of the Fill Density of MTA Produced by Two Placement Techniques. J Endod 2006; 32(5):456-9.
29. Araújo AC, Nunes E, Fonseca AA, Cortes MI, Horta MC, Silveira FF. Influence of smear layer removal and application mode of MTA on the marginal adaptation in immature teeth: a SEM analysis. Dent Traumatol 2013, 29(3):212-7.
30. Bustamante-Teixeira MT; Faerstein E; Latorre MR. Técnicas de análise de sobrevida. Cad Saúde Pública 2002; 18(30):579-94.

31. Brito-Júnior M, Faria e Silva AL, Quintino AC, Moreira-Junior G, Geber M, Camilo CC, Soares JA. Orthograde retreatment failure with extruded MTA apical plug in a large periradicular lesion followed surgical intervencion: case report. Gen Dent 2012; 60(2):e-96-100.