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ORIGINAL ARTICLE

Polymicrobial Leakage and Retention of MTA and Portland Cement in a Model of Apexification

Manoel Brito-Júnior[1], Maria Aparecida Barbosa de Sá[2], Sérgio Avelino Mota Nobre[3], André Luis Faria-e-Silva[4], Rodrigo Dantas Pereira[5], Carla Cristina Camilo[6], Frank Ferreira Silveira[7], Manoel Damião Sousa-Neto[8]

[1]Department of Dentistry, State University of Montes Claros, Montes Claros, MG, Brazil. 0000-0002-3652-4047
[2]Department of Dentistry, Pontifical Catholic University of Minas Gerais, Belo Horizonte, MG, Brazil. 0000-0002-6767-1007
[3]Department of General Biology, State University of Montes Claros, Montes Claros, MG, Brazil. 0000-0002-5507-0677
[4]Departmento of Dentistry, Federal University of Sergipe, Aracaju, SE, Brazil. 0000-0003-3846-4786
[5]Dental School, United Universities of the North of Minas, Montes Claros, MG, Brazil. 0000-0003-2538-1930
[6]Department of Dentistry, State University of Montes Claros, Montes Claros, MG, Brazil. 0000-0001-8168-9724
[7]Department of Dentistry, Pontifical Catholic University of Minas Gerais, Belo Horizonte, MG, Brazil. 0000-0002-4298-0540
[8]Department of Endodontics, School of Dentistry, University of Ribeirão Preto, Ribeirão Preto, SP, Brazil. 0000-0002-7696-7600

Author to whom correspondence should be addressed: Maria Aparecida Barbosa de Sá, Rua Lagoa Baixa, 324, Carmelo, Montes Claros, MG, Brazil. 39402-700 Phone: +55 38 99885-5323. E-mail: cidab.d.s@hotmail.com.

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Abstract

Objective: To evaluate the sealing capacity and retention of apical barriers made with mineral trioxide aggregate (MTA) and Portland cement (PC). **Material and Methods:** Fifty-six bovine incisors were sectioned 8 mm above and 12 mm below the cement-enamel junction. The root canal was enlarged with a diamond drill to create a standard 2.5 mm diameter opening. Apical sheets of 5 mm thickness were placed using white MTA-Angelus or white PC. Fifteen samples of each material were exposed to human saliva in a dual chamber apparatus and casting was evaluated at 30 days. Samples without apical barriers and fully sealed samples were used as positive and negative controls (n = 3), respectively. Data were analyzed by Fisher's exact test ($p<0.05$) after 3 periods: 1 to 10 days (P1); days 11 to 20 (P2); and days 21 to 30 (P3). Then, three 1 mm thick sections were obtained at the apical level of other root samples of each material (n = 10) and the push-out test was performed. **Results:** The leakage rates in P1, P2 and P3 were 60%, 73.3% and 100% for the MTA; and 73.3%, 86.7% and 100% for CP, with no significant difference between materials, regardless of the period analyzed. There were no significant differences between the bond strengths for both cements ($p>0.05$). **Conclusion:** Mineral trioxide aggregate and Portland cement apical barriers presented similar sealing ability and bond strength values.

Keywords: Endodontics; Tooth Injuries; Apexification; Dentition Permanent.

Introduction

Tooth traumas in children can result in injured pulp tissue and arrested root development with open apexes, and this clinical condition remains a challenge for clinicians. Partial pulpotomy, revascularization, apexogenesis, and apexification are therapeutic approaches indicated for these cases. Successful apexification or revascularization procedures in immature necrotic teeth have been reported [1-3].

During the apexification process, the immediate technique using an artificial apical barrier with mineral trioxide aggregate (MTA) has been recommended [4-6]. MTA presents proper biocompatibility and sealing ability, and the latter characteristic is essential for successful treatment [7,8]. Despite being advantageous, the elevated cost of MTA has not allowed its use in all levels of health attention. Portland cement (PC) presents chemical, physical, and biological characteristics similar to those of MTA and has a low cost [9-11]. Thus, the use of less expensive PC in apexification procedures has been considered clinically [12,13].

Different methods have been used to test the sealing ability of MTA or PC used as apical barriers including dye leakage [14,15], bacterial penetration [16-18], glucose leakage [15,19], capillary flow porometry [20] and fluid transport [21,22]. Some of these studies [20-22] indicate similar sealing properties of MTA and PC apical barriers, although the use of different methodologies makes it difficult to compare the results. In addition, the clinical and biological relevance of these methods is unclear [23,24]. Conversely, polymicrobial tracers closely approximate from clinical occurrences [24] but there is a lack of direct comparison of MTA and PC apical barriers using this method.

Another important property of endodontic materials is their retentive potential in the root dentin, which is frequently evaluated by the push-out test [25]. Considering the necessity to compact the filling material over the apical barrier in the final step of endodontic therapy, the apical plug must be able to remain bonded to root canal walls without displacement during the filling procedures. The retention of MTA used as an apical barrier [26] or placed in root dentin slices [27,28] has been reported; however, limited information is available about the retention of PC used as an orthograde apical barrier. Thus, the purpose of this study was to evaluate the sealing ability and retention of MTA and PC apical barriers in simulated immature bovine teeth. The null hypothesis tested was that both cements would present similar behaviors regarding the aforementioned material properties.

Material and Methods

Sample Selection and Preparation

Fifty-six bovine incisors presenting similar root dimensions were used in the present study. The buccolingual and mesiodistal dimensions of roots were measured, while roots presenting measurements more than 10% above the mean were discarded. The selected incisors were sectioned 8 mm above and 12 mm below the cemento-enamel junction using a low-speed diamond saw [29].

Pulp tissue was removed by the use of #60 Hedström files (Dentsply Maillefer, Ballaigues, Switzerland), and the root canal irrigated with 1% sodium hypochlorite (NaOCl). The root canals were enlarged coronal apically using a #3017 HL diamond bur (KG Sorensen, Barueri, SP, Brazil) to create a standard hole of 2.5 mm in diameter, followed by irrigation with 1% NaOCl. The remaining thickness of the root canal walls (buccal, palatine, mesial, and distal) near to root apex was standardized at 0.1–0.2 mm, measured with a digital caliper. After removing the smear layer with buffered 14.3% EDTA solution (Biodinâmica Química e Farmacêutica Ltda., Ibiporã, SP, Brazil) for 3 minutes, the root canals were irrigated again with 1% NaOCl and dried with paper points (Dentsply Maillefer, Ballaigues, Switzerland).

The open apices were sealed with a 5-mm-thick apical plug using white MTA (Angelus, Londrina, PR, Brazil) or white PC (Irajazinho, Votorantim Cimentos, Votorantim, SP, Brazil). The materials used for apical plugs (MTA and PC) were mixed at a power/liquid ratio of 3:1 and inserted into the root canal with an MTA carrier, followed by condensation with hand pluggers under ultrasonic vibration [17]. During this procedure, the samples were placed on the condensation-cured silicon impression material (Zetaplus dense, Zhermack, Badia Polsine, Italy), aiming to create artificial periradicular tissues and to prevent MTA extrusion. The teeth were radiographed to confirm the height and homogeneity of the apical plugs, followed by being stored at 37°C and 100% humidity for one week [30,31].

Polymicrobial Leakage Test

Thirty samples containing apical plugs (n = 15) were submitted for leakage testing. The absence of an apical plug (n = 3) and a totally sealed apex (n = 3) with three layers of cyanoacrylate adhesive (SuperBonder, Henkel LTDA, Itapeva, SP, Brazil) were used as positive and negative controls, respectively. A double-chamber apparatus proposed in previous studies [32,33] was used to verify the sealing ability of apical barriers.

Each tooth was individually inserted into a silicon tube (0.5 x 1.5 mm) used as a microbial reservoir, whereas the apical portion of the root remained out of the tube. The interface between the tooth crown and the tube was sealed with cyanoacrylate adhesive. The system (tooth inserted into a silicon tube) was sterilized using ethylene oxide gas and placed in a sterilized 50 mL glass flask containing 10 mL of sterile Brain Heart Infusion broth (BHI, Difco, Detroit, MI, USA). The interface between the silicon tube and the glass flask was also sealed with cyanoacrylate adhesive. The experimental model assembly procedures were performed in a laminar flow chamber to avoid contamination. Two milliliters of sterile 1% methylene blue dye were inserted into the tube up to the coronal portion of each sample to check the cyanoacrylate sealing efficiency. If the broth changed to a blue color, it meant the sealing was defective and the specimen was discarded.

The upper reservoirs of the chamber were subsequently filled with human saliva (20 mL) mixed in BHI broth in a 1:1 (v/v) ratio, which was renewed every 3 days. The top of the assembly was covered with Kraft paper and aluminum foil to avoid unintentional contamination. The entire apparatus was incubated at 37°C, and leakage was evaluated daily by checking the turbidity in the culture medium of the lower part of the chamber. The leakage was evaluated after three periods: days 1 to 10 (P1); days 11 to 20 (P2); and days 21 to 30 (P3).

Broth turbidity was scored as leakage, while color maintenance of the BHI medium was classified as the absence of leakage. The percentage values of leakage for each evaluated period were submitted to the Fisher exact test ($p<0.05$).

Push-out Test

The remaining 20 samples containing apical barriers ($n = 10$) were used for push-out testing. Each root was sectioned into three 1 mm thick slices, which were observed under a 40x magnification optical microscope to measure the dimensions of the barriers. Dimensions (radius and perimeter) of the upper and lower surfaces of the barriers were recorded to calculate the lateral area of each slice.

The slices were then placed in a pressure gauge in a mechanical testing machine (EMIC DL 2000, São José dos Pinhais, PR, Brazil) and a compressive load with a 5000N load cell at a rate of 0.5 mm / min in the apex-coronal direction, until the maximum force for the displacement of the barrier inside the root canal occurred. A 2.2 mm diameter stainless steel plunger was used to apply force. The bending strength values (MPa) were calculated by dividing the maximum load by the lateral area of the barrier. The bond strength values of all the slices of the same root were the mean values and the data were submitted to the T-test ($p<0.05$). The fractured specimens were observed with an increase of 40 × to classify the failure modes: Type I - adhesive at the interface between the barrier material and the dentin; Type II - cohesive in the material used as a barrier; and Type III - mixed failure.

Ethical Aspects

The study was evaluated and approved by the Research Ethics Committee of FUNORTE (Protocol No. 0289/09).

Results

For the polymicrobial leakage test, the positive control presented leakage in all specimens in the first 24 hours, while the negative control presented no samples with leakage. All samples of the MTA and PC apical barriers showed increasing leakage during the period of evaluation, whereas no significant difference was observed between MTA and PC regardless of the period analyzed (Table 1). Figure 1 illustrates the increase in samples with microbial leakage for each experimental group. For the push-out bond strength test, there was no difference between the material used as an apical barrier ($p=0.353$). This result is displayed in Figure 2. There was predominance of Type I failures for both MTA (23/29) and PC (19/24), while no Type II failures were observed.

Table 1. Absolute and relative frequency of the leakage according to the analyzed periods for each cement.

Period	Groups				p-value
	MTA		PC		
	N	%	N	%	
P1	9	60.0	11	73.3	0.700
P2	11	73.3	13	86.7	0.651
P3	15	100.0	15	100.0	1.00

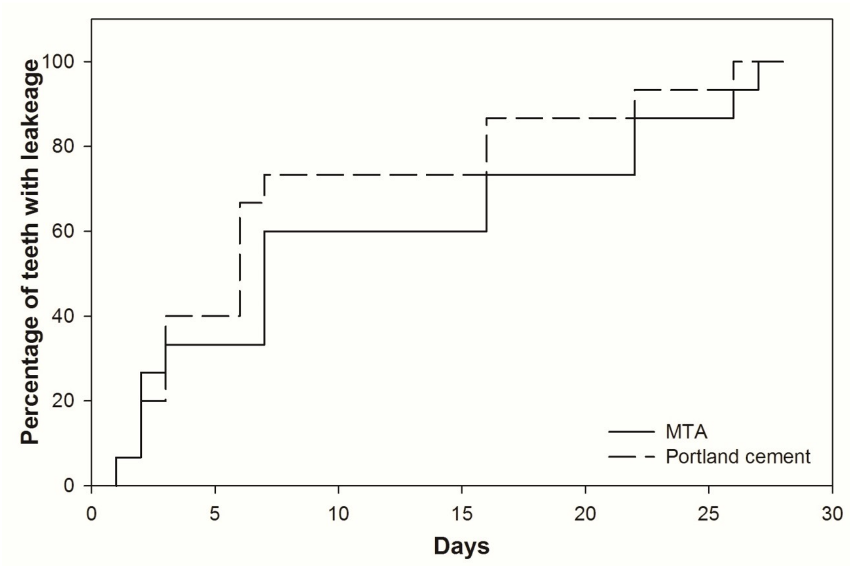

Figure 1. Percentage of teeth with leakage in each group during 30 days.

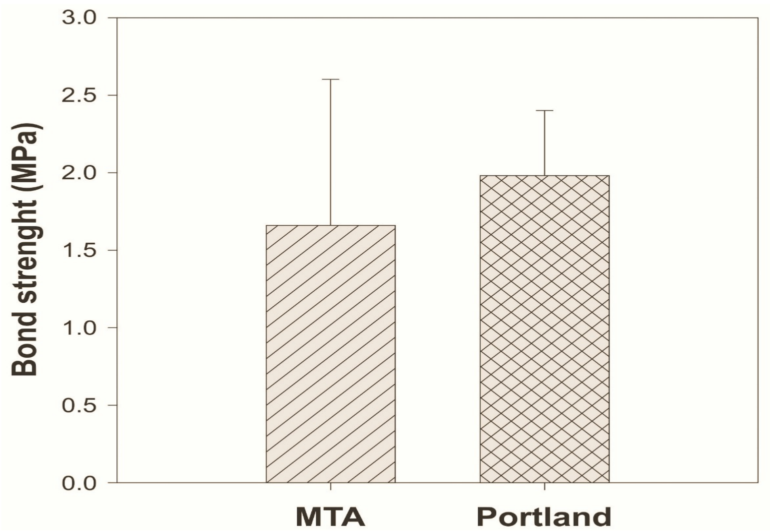

Figure 2. Push-out strength values (means) for each cement.

Discussion

Bovine teeth have been used as a substitute for human teeth in dental studies due to the difficulty of obtaining healthy extracted human teeth and the need to standardize the samples

[34,35]. Furthermore, bovine incisors commonly present large root canals, resulting in enlarged apexes, which simulates immature teeth [29]. Thus, the present experimental model resulted in a wide-open apex, challenging the sealing ability and the retention of the apical barriers evaluated.

Similar behavior of MTA and PC apical barriers was observed in the present study. Thus, the null hypothesis was accepted. It has been shown that the chemical components of both materials present a mixture of dicalcium silicate, tricalcium silicate, tricalcium aluminate, gypsum, and tetracalcium aluminoferrite [9,36]. The main difference between the materials is related to the presence of bismuth oxide in MTA, which provides radiopacity to this material [9,10]. Additionally, the particles of MTA and PC were macroscopically and microscopically identical when evaluated by scanning electron microscopy (SEM) and energy-dispersive X-ray analysis [37]. Furthermore, similar marginal adaption has been reported by SEM evaluation for both materials when used in root-end cavities [38]. Thus, these similarities between MTA and PC can explain the outcomes of the present study.

A polymicrobial method to evaluate the leakage through orthograde apical barriers was used in this study. This method presents more biological significance than other methods used for this same purpose [24,32,39,40]. Although the route of microbial leakage has not been traced histologically, which represents a methodological limitation [24]. The utility of the double-chamber leakage apparatus was confirmed in previous investigations [24,39,40].

Several factors, such as insertion and compaction of material, the thickness of barrier, and time of evaluation, can affect the sealing ability of apical barriers [38,41]. In the present study, a 5-mm-thick apical barrier, created using passive ultrasonic vibration, was performed. It has been demonstrated that these procedures improved the sealing ability of MTA [18,39,42,43]. Previous study showed that ultrasonically placed MTA apical barriers [18] showed that ultrasonically placed MTA apical barriers (5 mm) leaked in 20% and 60% of the samples after 60 and 180 days, respectively. Another investigation [44] also using 5-mm MTA apical plugs placed without ultrasonic vibration, showed no bacterial leakage after 70 days. In contrast, the present results showed that approximately 60% to 70% of the samples apically sealed with MTA or PC leaked after 10 days; this proportion reached 100% at 30 days. These differences can possibly be attributed to the apexification model and mainly to the method used to evaluate bacterial leakage. In this study, a polymicrobial leakage method was used, while the other studies [18,44] used single bacterial leakage methods, reducing the bacterial penetration in the apical barriers.

A closer contact between the material used as a barrier and the dentin walls is essential to improve the resistance to the displacement of apical barriers. In the present study, the smear layer removal in association with condensation procedures using ultrasonic vibration probably improved the marginal adaption of MTA and PC plugs as observed in the literature [43]. However, the absence of micromechanical retention (hybrid layer formation) and chemical bonding to the tooth substrate jeopardizes the retention of the apical barrier. These drawbacks can explain the prevalence of adhesive failures as well as relatively low mean push-out strengths for MTA (1.65 MPa) and PC

(1.98 MPa) plugs verified in the present study. Despite the absence of a correlation between bond strength and microleakage [45], low values of apical barrier retention to root canal can compromise its stability during the condensation of gutta-percha. Recently, it was shown that mixing MTA with propyleneglycol enhanced its bond strength to dentin [28]. It is not possible to affirm that the use of the same vehicle could improve the retention of apical barriers tested in the present study. Thus, further studies with alternative vehicles must be conducted to improve the retention of MTA and PC apical barriers in apexification models.

Conclusion

Mineral Trioxide Aggregate (MTA) and Portland cement apical barriers presented similar sealing ability and bond strength values.

Authors' Contributions: MBJ contributed to conception, writing, critical review and final approval of the article. MABS performed the experiment and wrote the manuscript. SAMN and ALFS designed the study and reviewed the manuscript. RDP, CCCM, FFS and MDSN performed the critical review and final approval of the article.

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Conflict of Interest: The authors declare no conflicts of interest.

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