A LABORATORY ASSESSMENT OF BACTERIAL LEAKAGE IN MTA APICAL PLUGS EXPOSED TO PHOSPHATE-BUFFERED SALINE

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ABSTRACT

This study evaluated the influence of the exposure of mineral trioxide aggregate (MTA) - with and without calcium chloride ($CaCl_2$) - to phosphate-buffered saline (PBS) on apical microleakage. Sixty root segments were divided into 4 experimental groups (n=15). Apical cavities were filled with MTA with or without $CaCl_2$, and the root canals dressed with a moistened cotton pellet or PBS: 1) MTA/cotton pellet; 2) MTA/PBS; 3) MTA+ 10%$CaCl_2$/cotton pellet; 4) MTA+10%$CaCl_2$/PBS. After 2 months, E. faecalis penetration was analyzed along the apical plugs. Samples were observed weekly for 70 days, and leakage was detected by turbidity of the medium in contact with the root segment. Teeth in the control groups (n=2) were either made completely impermeable or kept without an apical plug. The Kaplan–Meier method was used to analyze survival and the Log-rank test was used to compare the survival curves (p<0.05). All specimens in the positive control group showed evidence of leakage within 24h, while none in the negative control group showed leakage up to 70 days. There was no statistically significant difference among the experimental groups (p=0.102). The use of PBS as intracanal dressing may improve MTA sealing ability, but cannot prevent bacterial leakage. The addition of $CaCl_2$ to the MTA did not improve MTA sealing ability.

Key words: *apexification, dental leakage, endodontics, mineral trioxide aggregate.*

AVALIAÇÃO LABORATORIAL DA INFILTRAÇÃO BACTERIANA EM PLUGS APICAIS DE MTA EXPOSTOS AO TAMPÃO FOSFATO-SALINO

RESUMO

O presente estudo avaliou a influência da exposição do agregado de trióxido mineral (MTA) – com e sem cloreto de cálcio ($CaCl_2$) – ao tampão fosfato-salino (PBS) sobre a microinfiltração apical. Sessenta segmentos radiculares foram divididos em 4 grupos experimentais (n=15). As cavidades apicais foram preenchidas com MTA, com ou sem $CaCl_2$, e os canais radiculares receberam uma bolinha de algodão umedecida ou PBS, como medicação intracanal: 1) MTA/ bolinha de algodão umedecida; 2) MTA/PBS; 3) MTA+10% $CaCl_2$/ bolinha de algodão umedecida; 4) MTA+10%$CaCl_2$/PBS. Após 2 meses, a penetração de E. faecalis ao longo dos plugs apicais foi avaliada. As amostras foram observadas semanalmente durante 70 dias e a infiltração detectada através da turbidez do meio em contato com os segmentos radiculares. Dentes pertencentes aos grupos controle (n=2) foram mantidos completamente impermeáveis ou sem plug apical. A análise de sobrevivência e a comparação das curvas foram realizadas por meio dos testes Kaplan-Meier e Log-rank (p<0.05), respectivamente. Todas as amostras do grupo controle positivo apresentaram evidência de infiltração dentro de 24h, enquanto nenhuma amostra do grupo controle negativo apresentou infiltração ao longo dos 70 dias. Não houve diferença significativa entre os grupos experimentais (p=0.102). O uso do PBS como medicação intracanal pode melhorar a capacidade de selamento do MTA, mas não é capaz de impedir a infiltração bacteriana. A adição de $CaCl_2$ ao MTA não melhora sua capacidade de selamento.

Palavras-chave: *apexificação, infiltração dentária, endodontia, agregado de trióxido mineral.*

INTRODUCTION

Coronal leakage of microorganisms and their byproducts is considered a common reason for the failure of endodontic treatment. Therefore, any material intended to seal communications between the root canal and the periodontium[1-3] should be effective.

Mineral trioxide aggregate (MTA) used as an apical plug or as a retrofilling material rarely provides a totally efficient seal[4,5]. Some studies have shown that the interaction between MTA and phosphate-buffered saline (PBS)[6] or tissue fluid[7] leads to the formation of a layer with tag-like structures at the cement-dentin interface, which may positively

influence its sealing ability[3,8]. Despite the promising results obtained in these studies, a more recent investigation demonstrated that the use of PBS as intracanal dressing provided a slightly, though not significantly improved sealing[9].

It was believed that adding calcium chloride ($CaCl_2$) to MTA might have a positive influence on the biomineralization process[6] and contribute to its sealing ability; however, experiments found that the opposite was true, with the mixture allowing greater glucose leakage[9].

Due to the ongoing controversies regarding the benefits of PBS as an intracanal dressing and regarding the effect of adding $CaCl_2$ to MTA on microleakage, more evidence is needed. The bacterial leakage model, considered to be the most clinically relevant and biologically significant[10], might provide a more reliable result. The purpose of this study was to evaluate the influence of the exposure of MTA - with and without $CaCl_2$ - to PBS on apical microleakage using a bacterial penetration model.

MATERIALS AND METHODS

Sixty-four single-rooted, extracted human teeth were used under a protocol approved by the Ethics Committee for Research with Human Beings of the Federal University of Santa Catarina (protocol number 1861).

The procedures were performed as described by Almeida et al.[9]. The crowns were sectioned, and a 2-mm root tip resection was performed with a high-speed bur under water cooling, so that all root segments were about 12 mm long. The canals were cleaned and shaped using #1-5 Gates-Glidden drills in a crown-down fashion, and 1% sodium hypochlorite (NaOCl) was used for irrigation. A standardized open apex was created by retrograde preparation of the canal with a #6 Gates-Glidden drill (±1.50-mm diameter). The final canal rinse was performed with 17% EDTA followed by 1% NaOCl.

Apexification procedures

The root sections were randomly divided into 4 experimental groups (n = 15). The apical cavities were filled and the root canals dressed as described in Table 1.

MTA cement was mixed following the manufacturer's recommendations, and MTA+$CaCl_2$ was mixed following Bortoluzzi et al.[11]: 1 g MTA with 0.1 g $CaCl_2$ mixed with 0.18 mL H_2O.

The cement mixture was placed into the canal, condensed with moistened paper points, and compacted with pluggers (Dentsply, Tulsa Dental, Tulsa, OK, USA) to create a 4 mm-thick apical plug. Radiographs were taken of all root segments to ensure void-free MTA placement and plug thickness.

In Groups 1 and 3, following the manufacturer's recommendations, a cotton pellet moistened with distilled water was placed in the cervical region of each root segment, and replaced with a dry one after 24 h. In Groups 2 and 4, in order to favor the biomineralization process[6], the remaining canal space was filled with PBS (Dermus Farmácia Dermatológica e Cosmética Ltda, Florianópolis, SC, Brazil; pH = 7.2) as an intracanal dressing (Table 1).

All access openings were covered with cotton pellets and filled with temporary cement (Cimpat, Septodont Brasil Ltda, São Paulo, SP, Brazil). The root segments were placed in plastic vials containing floral foam moistened with 20 mL PBS, and stored for 2 months at 37°C.

After the experimental period, the external surfaces of all specimens were made impermeable with two layers of nail varnish, except for the 1 mm around the apical foramen. An apparatus with the root segment was mounted [similar to the model developed by de Leimburg et al.[12]], sterilized by ethylene oxide gas (ACECIL, Central de Esterilização Com. Ind. Ltda., Campinas, SP, Brazil), and adapted to a sterile 20-mL syringe containing 5 mL of Brain Heart Infusion broth (BHI), so that the most apical portion of each root segment was immersed in the culture medium. The syringe embolus allowed closure of the apparatus, which was kept in an oven at 37°C for 4 days to confirm sterilization.

Table 1: Groups, Materials Used to Form the Apical Plug and Intracanal Dressing.

Group	Apical plug	Intracanal dressing
1	MTA*	Moistened cotton pellet
2	MTA*	PBS
3	MTA*+$CaCl_2$#	Moistened cotton pellet
4	MTA*+$CaCl_2$#	PBS

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# Vetec Química Fina, Rio de Janeiro, RJ, Brazil.

Bacterial leakage test

For the leakage assay, a standard strain of *Enterococcus faecalis* (ATCC 29212) was used. Previously to testing, the *E. faecalis* counts in the BHI were determined by decimal dilutions. Aliquot portions were plated on the surface of trypticase soy agar (TSA) (Difco Laboratories, Becton Dickinson and Company, Franklin Lakes, NJ, USA) and incubated at 37ºC for 24 h. After the incubation period, the number of colony forming units (CFU mL-1) was determined. For the bacterial leakage test, 500 µL aliquots of *E. faecalis* were transferred to the upper portion of the pipette connected to the root segment. Every 7 days during the experimental period, the BHI inoculated with *E. faecalis* was replaced with a new 500 µL aliquot of sterile BHI. The aliquot removed was tested to confirm bacterial viability. The number of leaking samples for each group was observed weekly for 70 days. Leakage was detected by turbidity of the BHI medium in contact with the apical portion of the root segment.

For the positive control group, two root segments without apical plugs were used. For the negative control group two root segments with apical plugs were made completely impermeable by the application of two layers of nail varnish. Additionally, one specimen of each experimental group was used as negative control and inoculated with BHI without *E. faecalis*.

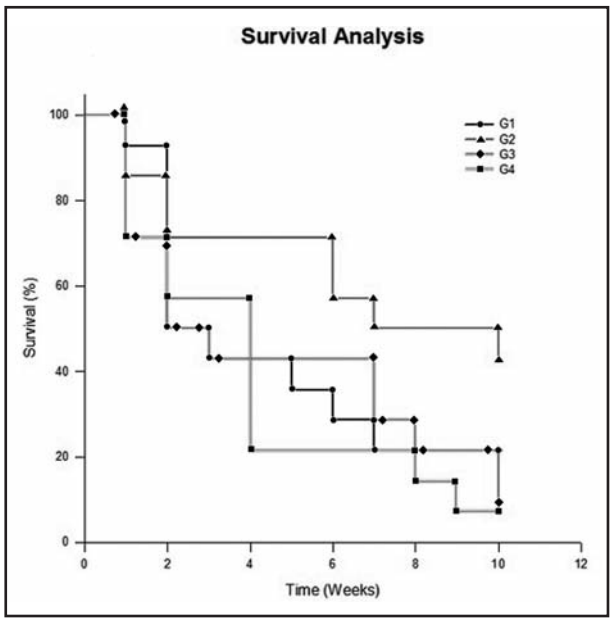

Fig. 1: Plot of Kaplan-Meier survival analysis for bacterial leakage in the experimental groups over the 70-day period.

Statistical analysis

The Kaplan-Meier method was used to estimate survival curves for each experimental group. Log-rank testing was used to compare the survival curves at the 5% significance level.

RESULTS

Control groups

All specimens in the positive control group exhibited leakage within 24 h, and the inoculums were confirmed to contain *E. faecalis,* while none in either of the negative control groups showed leakage up to 70 days.

Experimental groups

Fig. 1 shows the survival curves. Over the 70 days there was no statistically significant difference among groups ($p = 0.102$).

DISCUSSION

Several *in vitro* methodologies are available for evaluating the quality of the apical seal provided by apical plugs, such as glucose penetration[9], fluid filtration techniques[13] and bacterial leakage[14]. Due to the controversial history of the reproducibility and clinical relevance of these methods, the issue of microleakage has been intensively discussed[15,16]. However, among all *in vitro* methods, the bacterial leakage test reflects clinical reality, since it uses bacteria, which are etiologic agents of apical periodontitis, as markers[17]. The 70-day experimental period was carried out based on the methods used in previous bacterial leakage studies[18,19]. The medium in contact with the root segments of the negative control groups showed absence of turbidity up to the end of the experiment, and confirmed the suitability of the apparatus and the absence of contamination. The culture medium in contact with the root segments of the positive control group, without MTA apical plugs, became turbid within the first 24 h, confirming that microorganisms might reach the apical region in the absence of apical plugs.

Monitoring of the experimental groups up to 70 days showed that, regardless of the addition of $CaCl_2$ to MTA or the use of PBS as intracanal dressing, most of the apical plugs did not prevent bacterial leakage. A possible reason for this was contemplated in a similar study using the glucose leakage method[9]. Through-and-through voids in the body of the material or the cement-dentin interface[20]

as well as interconnected pores[21] probably served as a route for bacterial penetration.

It is worth noting that under our experimental conditions, the interaction of PBS as intracanal dressing with MTA apical plugs provided better sealing, although it was not significantly different. Despite using different leakage methodologies, recent investigations have demonstrated similar results when MTA was kept in contact with PBS[3,8,9]. The most acceptable explanation is based on the formation of carbonated apatite at the biomaterial-dentin interface[6]. The association of PBS as intracanal dressing that diffused through the apical barrier encourages the occurrence of the biomineralization process along the MTA apical plugs[22], probably reducing the leakage.

Despite its benefits regarding setting time and pH[11], the addition of $CaCl_2$ to MTA jeopardized its sealing ability, regardless of the use of PBS as intracanal dressing. It was noticed that a high number of MTA+ $CaCl_2$ apical plugs (28.57%) allowed bacterial leakage in the first week and by day 70 almost all the samples had leaked (92.85%). These results agree with a recent leakage study[9]. The addition of $CaCl_2$ to MTA promotes greater absorption of water by the cement, leading to the formation of capillary pores and loss of sealing ability[23].

The findings of this study confirm that the use of PBS as intracanal dressing may improve MTA sealing ability, but cannot totally prevent bacterial leakage. The addition of $CaCl_2$ to the MTA did not improve its sealing ability. Although there are still controversies in microleakage studies, it is very useful to know the behavior and effectiveness of the MTA in different situations.

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