

SEALING ABILITY OF MTA-ANGELUS WITH PROPYLENEGLYCOL IN FURCAL PERFORATIONS

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ABSTRACT

This study evaluated the sealing ability of gray MTA-Angelus mixed with propyleneglycol in furcal perforations using a bacterial leakage test. Furcal perforations were created in 30 human mandibular molars using a size 3 round bur. The samples were divided randomly into 2 experimental groups (n=10) according to the mixing agent. In G1, the MTA powder was mixed with propyleneglycol, while distilled water was used in G2. A 3:1 powder-liquid ratio was used for both groups. The MTA was placed in the perforation with an MTA carrier and condensed with hand pluggers. Non-repaired (n=5) and totally sealed (n=5) perforations served as positive and negative controls, respectively. Bacterial leakage was assessed daily for 30 days in a double-chamber apparatus with *Enterococcus faecalis*. Data were analyzed using Fisher exact test ($p<0.05$) for three leakage periods: 1st to 10th day (P1); 11th to 20th

day (P2); and 21st to 30th day (P3). The positive control presented leakage in all specimens within the first 24 hours, while no leakage was observed in the negative control during the experimental period. Leakage was observed in five (50%) of the 10 samples of the propyleneglycol group (G1) and seven (70%) of the distilled water group (G2) by the 20th day, without significant difference between the groups in periods P1 and P2 ($p=0.137$). The leakage was significantly lower for G1 than G2 in period P3 (50% versus 100%, respectively, $p=0.016$). In this single aerobic bacterial leakage method, the use of propyleneglycol as a vehicle for gray MTA-Angelus increased its sealing ability in furcal perforations at the end of the 30-day experimental period.

Key words: mineral trioxide aggregate, dental pulp cavity, *Enterococcus faecalis*.

CAPACIDADE DE SELAMENTO DO MTA-ANGELUS COM PROPILENOGLICOL EM PERFURAÇÕES DE FURCA

RESUMO

Este estudo avaliou a capacidade de selamento do MTA-Angelus cinza manipulado com propilenoglicol em perfurações de furca usando um teste de infiltração bacteriana. Perfurações de furca foram criadas em 30 molares inferiores usando uma broca esférica número 3. As amostras foram divididas aleatoriamente em dois grupos experimentais (n=10) de acordo com o agente de manipulação. Em G1, o pó do MTA foi manipulado com propilenoglicol enquanto que a água destilada foi utilizada no G2. A proporção pó-líquido de 3:1 foi usada para ambos os grupos. O MTA foi colocado na perfuração com uma porta-MTA e condensado com condensadores manuais. Perfurações não-reparadas (n=5) e totalmente impermeabilizadas (n=5) serviram como controle positivo e negativo, respectivamente. A infiltração bacteriana foi verificada diariamente durante 30 dias em um aparato de dupla câmara com *Enterococcus faecalis*. Os dados foram analisados pelo teste exato de Fisher

($p<0,05$) para três períodos: 1° ao 10° dia (P1), 11° ao 20° dia (P2) e do 21° ao 30° dia (P3). O controle positivo apresentou infiltração em todas as amostras nas primeiras 24 horas, enquanto nenhuma infiltração foi observada no controle positivo durante o período experimental. Infiltração foi observada em 5 (50%) das 10 amostras do grupo do propilenoglicol (G1) e 7 (70%) do grupo da água destilada no 20° dia, sem diferença significativa entre o grupos nos períodos P1 e P2 ($p=0,0137$). A infiltração foi significativamente menor para G1 que para G2 no período P3 (50% versus 100%, respectivamente, $p=0,016$). Neste método de infiltração de apenas uma bactéria aeróbica, o uso do propilenoglicol como veículo para o MTA-Angelus cinza aumentou sua capacidade de selamento em perfurações de furca no final do período experimental de 30 dias.

Palavras chave: agregado trióxido mineral, cavidade pulpar, *Enterococcus faecalis*.

INTRODUCTION

Furcation perforation represents communication through the pulp chamber floor in multi-rooted teeth. Its etiology is related to pathologic processes (caries or resorption) or to procedural errors during access to root

canals¹. Successful treatment of these perforations depends on adequate sealing by using a material with desirable physicochemical and biological properties^{2,3}. Mineral trioxide aggregate (MTA), commercially available as ProRoot (Dentsply Tulsa Dental, Tulsa, OK,

USA) and MTA-Angelus (Angelus Soluções Odontológicas, Londrina, PR, Brazil), is a hydrophilic biomaterial that presents favorable characteristics for furcal perforation repair²⁻⁶. The MTA is a white or gray powder constituted of silica tricalcium, aluminum tricalcium, and oxide tricalcium and silicate oxide, and is similar to Portland cement⁷. MTA also contains bismuth oxide, a component responsible for its radiopacity, which facilitates radiographic investigations^{3,8}. In addition, it has good biocompatibility⁹, capacity to induce periodontal ligament repair¹⁰ and mineralized tissue formation¹¹.

Despite these advantages, MTA is granulose and has low cohesive properties, making it difficult to handle, particularly with distilled water¹². Thus, alternative vehicles have been suggested for this material. The ProRoot MTA manipulated with propyleneglycol appears to be more easily inserted in the root canals of dogs' teeth than when distilled water was used for this purpose¹³.

Different methods have been used to test the sealing ability of MTA in furcal perforations, such as dye leakage^{14,15}, bacterial penetration^{4,5}, fluid transport^{6,16} and protein leakage¹⁷. As regards the use of MTA-Angelus brand as a furcation repair material, most of the available information originates from experimental models using dye leakage^{14,18}. However, there is a lack of correlation between bacterial and dye leakage¹⁹. Thus, it is important to test the MTA-Angelus as furcation perforation repair material using a bacterial leakage model.

The aim of this *ex vivo* study was to test the hypothesis that the sealing ability of gray MTA-Angelus mixed with propyleneglycol works better than mixed with distilled water in furcal perforations, evaluated by means of the bacterial leakage test.

MATERIAL AND METHODS

Sample selection and preparation

This study was approved by the Research Ethics Committee of the North of Minas United Schools – Funorte (Protocol 0289/2009). Thirty human mandibular molars with intact crowns and complete root formation, within 3 months after extraction, were used in this study. Teeth with crown destruction and fractures were excluded. The teeth were stored in a 1% thymol solution until they were used, when they were washed thoroughly under running water.

Coronal access was initially performed with a #1557 tapered carbide bur (S.S. White Dental products, Rio

de Janeiro, RJ, Brazil) at high speed, followed by cavity wall finishing with an Endo-Z bur (Dentsply/Maillefer, Ballaigues, Switzerland). In all the teeth, root canal orifices were sealed using the adhesive technique. 37% phosphoric acid was applied for 15 seconds, followed by thorough rinsing with water and drying with absorbent paper. The adhesive system Prime & Bond 2.1 (Dentsply, Petrópolis, RJ, Brazil) was applied in two consecutive coats with a disposable microbrush. The solvent was removed with a slight air stream and the adhesive was light-cured for 20 seconds. Composite resin Filtek Z-100 (3M-ESPE, St. Paul, MN, USA) was inserted in a single increment at the entrance of each root canal and light-cured for 60 seconds. Afterwards, all teeth were sectioned through the middle third using a diamond disc (KG Soresen, Barueri, SP, Brazil) and protected with two layers of cyanoacrylate adhesive (SuperBonder, Loctite, Itapeva, SP, Brazil). The furcation perforations were performed in the center of the pulp chamber floor, using a # 3 round bur (Dentsply-Maillefer) at low speed.

Perforation sealing

To seal the perforation the teeth were placed on the condensation-cured silicon impression material (Zetaplus dense, Zhermack, Badia Polsine, RO, Italy), aiming to create artificial periradicular tissues and preventing MTA extrusion. Teeth were randomly divided into 2 experimental groups, according to the vehicle used for gray MTA-Angelus (Angelus Soluções Odontológicas, Londrina, PR, Brazil) handling: propyleneglycol (n=10) and distilled water (n=10). The propyleneglycol was obtained from a manipulation pharmacy (Nature Farm, Montes Claros, MG, Brazil). In the positive control (n=5) the perforations were left without sealing and in the negative control (n=5) the specimens were completely sealed with two layers of cyanoacrylate adhesive (SuperBonder, Loctite).

The MTA powder was mixed with sterile distilled water in the proportion of 3:1, according to the manufacturer's recommendations. The same powder/liquid proportion was used with propyleneglycol. In each group the material was placed in perforation sites by using an MTA carrier. A # 4 Schilder's plugger (Odous, Belo Horizonte, MG, Brazil) was used to condense the MTA. After sealing the perforation, the samples remained at 37°C and 100% humidity for at least 7 days to allow the MTA to set completely.

Bacterial leakage test

A double-chamber apparatus for evaluating bacterial leakage, proposed in a previous study⁵, was used to verify the sealing ability of MTA. Each tooth was individually inserted into a silicon tube (0.5 x 1.5 mm) that worked as a bacterial reservoir, with the furcation region projected toward the outer part of the tube. The interface between the tooth crown and the tube was sealed with cyanoacrylate adhesive (SuperBonder, Loctite). The system (tooth inserted into a silicon tube) was sterilized using ethylene oxide gas and placed in a sterilized 50ml glass flask containing 10 ml 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 (SuperBonder, Loctite). The experimental model assembly procedures were performed in a laminar flow chamber to avoid contamination. Two milliliters of sterile 1% methylene blue dye were put 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 that the sealing was defective and the specimen was discarded. The upper reservoirs of the chamber were subsequently filled with 500µL bacterial suspension containing *Enterococcus faecalis* ATCC 1092 at concentration of 1×10^8 UFC/mL. Then the top of the assembly was covered with Kraft paper and aluminum foil to avoid unintentional contamination. The entire apparatus was incubated aerobically at 37°C, and bacterial leakage was evaluated daily by checking the turbidity in the culture medium of the lower part of the chamber. The bacterial inoculation was renewed every 3 days, for 30 days. Three periods were structured according to the time interval in which the bacterial leakage occurred: 1st to 10th day (P1); 11th to 20th day (P2) and 21st to 30th day (P3). If turbidity was observed, a 10µl aliquot was taken from the contaminated BHI medium, streaked onto a plate containing Esculin Agar (Himedia Laboratories, Mumbai, India) and incubated for 24 hours. This medium is used for bacterial culture and identification of bacteria that can hydrolyze esculin (in this case, the *Enterococcus* genus) and produce hydrogen sulphide, which results in a dark color. The purpose of the procedure was to identify the leakage of *E. faecalis* only, and to prove the absence of contamination by the other microorganisms.

Statistical analysis

Data were analyzed using the Fisher exact test ($p < 0.05$).

RESULTS

The positive control group presented leakage in all specimens in the first 24 hours, while the negative control group presented no sample with leakage at the end of 30 days. In the samples that presented leakage, only the *E. faecalis* bacteria were identified, thus ensuring the absence of contamination by other microorganisms. According to Table 1, leakage was observed in five (50%) of the 10 samples of the propyleneglycol group and seven (70%) of the distilled water group in periods P1 and P2 (initial evaluation periods), without significant differences between the groups ($p = 0.137$). However, in period P3 (final evaluation period), there was significant difference between the experimental groups. Leakage was significantly less in the propyleneglycol group (50%) than in the distilled water group (100%) ($p = 0.016$). Fig. 1 shows the increase in samples with bacterial leakage during the experimental period for each group.

Table 1: Absolute and relative frequency of the bacterial leakage according to the analyzed periods, in each group.

Groups	Leakage Period*								
	P1		P2		P3				
	n	%	n	%	n	%	n	%	
Propyleneglycol (n=10)	5	50	a	5	50	a	5	50	a
Distilled water (n=10)	6	60	a	7	70	a	10	100	b

A different letter in the same column means significant differences between the groups (Fisher exact test, $p < 0.05$).
*P1, P2 and P3: bacterial leakage in the period from the 1st to 10th day; 11th to 20th day; and 21st to 30th day, respectively.

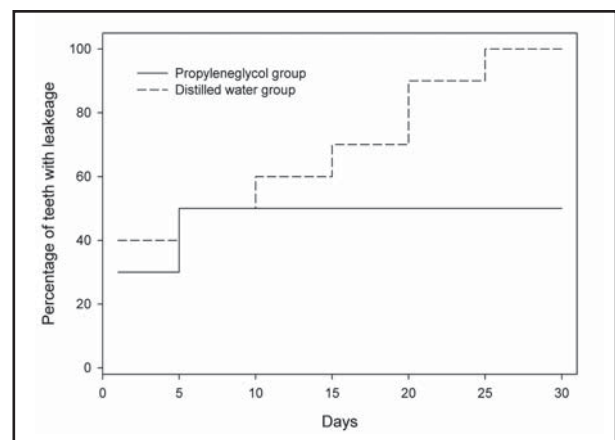


Fig. 1: Percentage of teeth with bacterial leakage in propyleneglycol and distilled water groups during the experimental period.

DISCUSSION

The hypothesis tested was partially supported by results. The results of the present study indicated that the gray MTA-Angelus mixed with propyleneglycol and water presented similar behavior when observed for 10 and 20 days. However, MTA mixed with propyleneglycol resulted in significantly lower bacterial leakage than MTA mixed with water at the end of 30 days.

It has been suggested that the bacterial leakage experimental model most closely approximates clinical reality⁴. In this study the controls behaved as expected, confirming the validity of the double chamber leakage apparatus used, as verified in a previous study⁵. The microorganism tested in this study, *E. faecalis*, is an important pathogen in endodontic infections, and has been used in bacterial leakage studies²⁰. Furthermore, the number of specimens used in the present study was similar to other studies that also used human teeth^{5,20}.

Investigations using gray ProRoot MTA to seal furcal perforations in extracted human molars showed bacterial leakage of approximately 25 to 50%^{4,5}. In the present study, it was verified that at the end of 30 days, there was 50% and 100% bacterial leakage when the gray MTA-Angelus was mixed with the propyleneglycol and distilled water, respectively. Thus, greater bacterial leakage was verified mainly in the distilled water group. MTA-Angelus and ProRoot MTA have various similarities, including sealant ability in furcal perforations tested by fluid transport⁶ and dye penetration¹⁷. However, it should be pointed out that MTA-Angelus particles have relatively low sphericity and a wide size distribution, and they are less homogeneous than ProRoot MTA²¹. This characteristic of MTA-Angelus may have made it more difficult to manipulate and insert into the perforation sites, negatively affecting the sealing ability

by deficiency of the marginal adaptation of the material. One study demonstrated that the resistance of MTA-Angelus to dye penetration in furcation perforations was only observed with the use of an internal collagen matrix. This suggests that the matrix was important for adapting the material to the cavity in order to prevent marginal microleakage¹⁴.

Another possible explanation for the significantly higher bacterial leakage in the distilled water group is the solubility of gray MTA-Angelus after setting, when handled in accordance with the powder-liquid ratio recommended by the manufacturer. When MTA powder is hydrated, it forms a colloidal gel, which becomes a rigid structure when it solidifies. The powder/liquid ratio can influence the characteristics of the mixture. For example, the MTA becomes more porous and soluble if the water-to-powder ratio is increased²². It has been reported that gray MTA-Angelus is more soluble than a type of Portland cement, up to 28 days after its handling with distilled water²³. Under similar experimental conditions the gray ProRoot MTA showed low solubility²⁴. Propyleneglycol has been used in endodontics as vehicle for calcium hydroxide intracanal dressings²⁵ and has been used with ProRoot MTA as filling cement in the root canals of dogs' teeth¹³. Propyleneglycol is an odorless, viscous liquid and is soluble in water. It has solvent, plasticizing and humidifying action. The better performance of the gray MTA-Angelus handled with propyleneglycol in the final evaluation period was probably due to the better homogeneity and decrease in cement porosity. Moreover, the mixture of propyleneglycol-MTA could have favored a greater expansion of material while setting, which is one of the possible reasons for the good sealing ability of MTA²⁶. Additional research is needed to clarify the physico-chemical properties of gray MTA-Angelus when handled with propyleneglycol.

CORRESPONDENCE

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