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# Effect of calcium hydroxide as intracanal medication on the apical sealing ability of mineral trioxide aggregate (MTA): an *in vitro* apexification model

*Efeito da medicação intra-canal de hidróxido de cálcio na capacidade seladora do mineral trióxido agregado (MTA): um modelo de apicificação in vitro*

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## Abstract

**Objective** – To evaluate the effect of calcium hydroxide premedication on the apical seal of White MTA, placed as an apical barrier in permanent teeth with simulated immature apices. Furthermore, we intended to compare potential changes, under the influence of calcium hydroxide, in the apical seal of MTA over time. **Methods** – Thirty-four single-rooted extracted teeth were prepared in order to simulate a divergent open apex. Two experimental groups of 10 teeth were created: group 1 (G1) and group 2 (G2) with and without calcium hydroxide intracanal medication previous to the placement of MTA apical plug. Two control groups, positive and negative, each with 7 teeth were created. On the 7<sup>th</sup> and on the 28<sup>th</sup> day after placement of the MTA apical plug, the apices of the teeth were submersed in a solution of sodium pertechnetate (<sup>99m</sup>TcO<sub>4</sub>Na) for 3 hours. The radioactivity was measured using a gamma camera. **Results** – Results revealed statistically significant differences between the 2 control groups and the 2 experimental groups with respect to the microleakage. Within the experimental groups no statistically significant differences were found; nor between the two observed periods. **Conclusion** – Based on the results obtained in this study, it was concluded that intracanal medication with calcium hydroxide did not affect the sealing ability of WMTA, placed as an apical plug, neither on the 7<sup>th</sup>, nor on the 28<sup>th</sup> day.

**Descriptors:** Dental cements; Tooth apex; Calcium hydroxide; Endodontics; Root canal filling materials

## Resumo

**Objetivo** – Avaliar o efeito do hidróxido de cálcio como medicação intracanal, no selamento apical do MTA branco, colocado como uma barreira apical em dentes permanentes com ápices imaturos simulados. **Métodos** – Trinta e quatro dentes unirradulares extraídos foram preparados para simular um ápice aberto divergente. Foram separados em dois grupos experimentais (n=10): G1 e G2 com e sem medicação intracanal de hidróxido de cálcio antes da colocação do tampão apical com o MTA e dois grupos controle, positivos e negativos (n=7 em cada). Em 7 e 28 dias após a colocação do tampão apical de MTA, os ápices dos espécimes foram submersos em uma solução de pertechnetato de sódio (<sup>99m</sup>TcO<sub>4</sub>Na) por 3 horas. A radioatividade foi medida usando uma câmara gama. **Resultados** – Os resultados revelaram diferenças estatisticamente significativas entre os grupos controle e os grupos experimentais com relação a microinfiltração. Não houve diferença entre os grupos experimentais nos dois períodos. **Conclusão** – Com base nos resultados obtidos neste estudo, concluiu-se que a medicação intracanal com hidróxido de cálcio não influenciou na capacidade seladora do MTA.

**Descritores:** Cimentos dentários; Ápice dentário; Hidróxido de cálcio; Endodontia; Materiais restauradores do canal radicular

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## Introduction

The occurrence of pulp necrosis in an immature permanent tooth caused by trauma or by pulpal disease can be considered an endodontic challenge due to several inherent characteristics of these teeth<sup>1-2</sup>.

The immature permanent tooth has an incomplete root formation, characterized by unreached final root length and thus an unfavourable crown/root ratio; thin root dentinal wall; wide canal space; apical divergence, absence of an apical constriction and an open apex<sup>2</sup>.

The traditional techniques of mechanical and chemical disinfection of root canals in teeth with an incomplete root formation are limited. Instrumentation increases the risk of weakening the thin dentinal walls. The divergent apical architecture and the lack of an apical stop make complete debridement and control of the filling nearly impossible<sup>3</sup>.

Given its high success rate the treatment of choice for these cases has been considered the placement of a mineral trioxide aggregate (MTA) apical plug<sup>4-8</sup>. Before placing the MTA apical barrier, the manufacturer recommends the use of calcium hydroxide [Ca(OH)<sub>2</sub>] for one week as intracanal dressing and its subsequent removal<sup>9</sup>. The use of calcium hydroxide has been considered an important step in the reduction of the intracanal microbial flora<sup>10</sup>. Because of the detrimental effects of an acidic pH, caused by the inflammation of periapical tissues, on various physical properties of MTA, it could be advisable to delay the placement of the MTA plug to a second session, using Ca(OH)<sub>2</sub> as inter-appointment intracanal medication in order to achieve additional disinfection and neutralization of an acidic environment<sup>2,11</sup>.

Before placing the apical plug, the removal of Ca(OH)<sub>2</sub> has been recommended to allow proper adhesion between the filling material and the root dentin<sup>12-13</sup>. However, it has

been shown that complete removal of calcium hydroxide from the root canal walls is very difficult to achieve if not impossible, at least with the techniques so far available<sup>1,13-16</sup>. The question that naturally arises is whether the remaining calcium hydroxide may affect the sealing ability of the MTA apical plug and consequently the results of the treatment.

The aim of this experimental *in vitro* study was: to evaluate the influence of the calcium hydroxide paste, applied as dressing over a period of 7 days, on the apical sealing of the MTA apical barrier placed in permanent teeth with simulated immature apices and to compare potential changes of the apical sealing of the MTA plug over time, under the influence of this dressing. For this purpose it was used an experimental model of a tooth with open apex, and the infiltration was assessed on the 7<sup>th</sup> and the 28<sup>th</sup> day after placing the MTA apical barrier, using <sup>99m</sup>TcO<sub>4</sub>Na as a tracer.

## Methods

Thirty-four single-root extracted human teeth were used. The inclusion criteria were teeth with mature apices, single canal, with no caries lesions, visible fractures nor fissures, without any restoration, with no signs of internal or external resorption, without calcification and without pronounced apical curvature. Preoperative radiographs served to confirm these criteria. The cleaning of the external surface, including the removal of soft tissues and calculus were carried out with the aid of Gracey curettes. The crowns of all teeth were removed with a cylindrical diamond drill, mounted on turbine with refrigeration, in order to obtain 17.0 mm in length. Then 2.0 mm of the roots tip were removed using also a cylindrical diamond drill, mounted on turbine with refrigeration, to obtain a final length of 15.0 mm. The teeth had been kept in a floral foam moistened with 0,9% sodium chloride (NaCl) (saline solution Braun®) to create an environment similar to the existent *in vivo*, with the intent to simulate the soft periapical tissues. The coronal access was performed by a round bur and the patency of the root canals was established with a #15 K-file. A divergent open apex was created by retrograde preparation with ProFile Orifice Shaper® OS#1 (05/20), OS#2 (06/30) (Dentsply Maillefer, Ballaigues, Switzerland) inserted 8.0 mm and operated on a X-SMART™ (Dentsply Maillefer, Ballaigues, Switzerland) electric motor with constant speed (300rpm) and torque (1N/cm) control. The root canals were biomechanically prepared by instrumentation and irrigation with sodium hypochlorite (NaOCl) at 1.0%. Orthograde preparation was carried out with ProFile® instruments (04/20-35) to D15 (Dentsply Maillefer, Ballaigues, Switzerland). Following the canal preparation thirty-four root segments were randomly divided into four groups: two experimental groups respectively with 10 teeth each and two control groups with 7 teeth each. In the positive control group (PG) (n=7), the samples were left unfilled and were double coated with nail varnish except for the apical 1.0 mm. In the negative control group (NG) (n=7) a 4.0 mm apical barrier of MTA was placed into the canals without any previous medication and all root segments were coated with two coats of nail varnish, including the apices. In

G1 (n=10) a 4.0 mm apical plug of MTA was placed into the canals previously medicated with calcium hydroxide for a period of 7 days and the root segments were double coated with nail varnish except for the apical 1.0 mm. In G2 (n=10) a 4.0 mm apical plug of MTA was placed into the canals without prior calcium hydroxide use and the root segments were double coated with nail varnish except for the apical 1.0 mm.

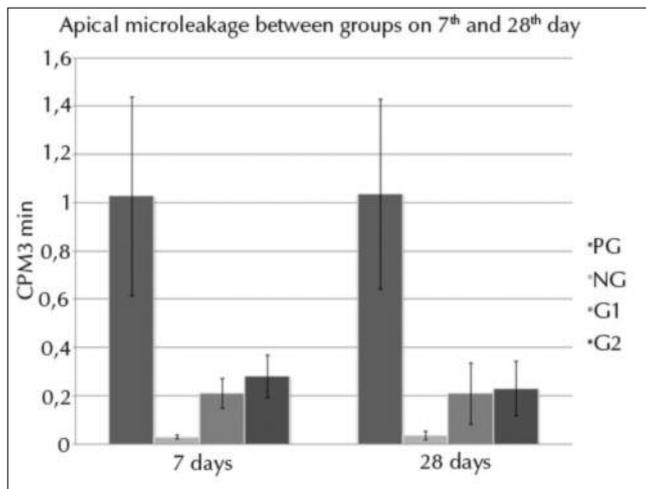
After drying the canals of the samples of the group (G1), calcium hydroxide paste (CalciCur® – Voco GMBH, Cuxhaven, Germany) was applied with a size 30 K-file. After filling the canal with the medication, a dry cotton pellet was placed at its entrance and the canal space was sealed with a temporary restorative material (Cavit® – 3M ESPE, Seefeld, Germany) put in the access cavity. After one week the dressing was removed through irrigation with NaOCl 1.0% and a #30 K-file and the canals were drying with paper points, orthograde mineral trioxide aggregate (MTA) – Pro Root MTA® (White MTA) (Dentsply Maillefer, Ballaigues, Switzerland) apical plugs with a thickness of 4.0 mm were placed the root canals of all groups except positive group, whose root segments were left unfilled. The mixture of MTA was performed according to the manufacturer's instructions with a powder/distilled water ratio of 3/1. The carrier MAP System (Micro Apical Placement System) (Produits Dentaires SA, Vevey, Switzerland) was used for placement of MTA into the canals subsequently condensed with Schilder pluggers (Dentsply Maillefer, Ballaigues, Switzerland), with stop placed at 11.0 mm. MTA placement was performed under an operating microscope (Leica CLS® 150MR) by the same operator. Afterward a cotton pellet embedded in 0.9% NaCl was put at the entrance of the canal and temporary restorative material Cavit® (3M ESPE, Seefeld, Germany) was used to seal the access cavity and samples were stored in 100% relative humidity for 7 days to allow MTA to fully set.

On the 7<sup>th</sup> and 28<sup>th</sup> day after placement of the MTA two coats of nail polish were applied to the external surface of each root except for the apical 1.0 mm, unlike the negative control group, where the root ends were also sealed with nail polish. The apical region (3.0 mm) of the samples were submersed into a solution of sodium pertechnetate (<sup>99m</sup>TcO<sub>4</sub>Na), placed in radioimmunoassay tubes, during 3 hours. Afterward, the removal of the radioisotope solution present on the outer surface of the root segments followed, by keeping them under running water and removing layers of varnish with a scalpel. Prior to new immersion on 28<sup>th</sup> day, the samples were again coated with nail varnish and its removal preceded the placement of the root segments before the gamma camera. The evaluation of the apical microleakage was determined indirectly by capturing the radioactivity emitted by the samples through the gamma camera (GE 400 AC, Milwaukee, USA) controlled by an acquisition computer (GenieAcq). For each tooth a static image was acquired for three minutes for a 512x512 matrix size. Regions of interest (ROIs) in each image were drawn over each tooth, to obtain the total counts and the counts per minute (cpm). The procedure was repeated at 7 and 28 days. The mean counts obtained in each image were used to determine the degree of infiltration.

Statistical analyses were performed at a significance level of 5%, using the software SPSS, version 19. The statistical tests applied were nonparametric. To compare the groups each time a Mann-Whitney test with multiple comparisons adjusted was used, and to compare the data obtained in different periods for each group a Wilcoxon test was applied.

## Results

Statistical analysis showed statistically significant differences between the control groups and the experimental groups. Graph 1 presents a general comparison of the radioactivity measured on the 7<sup>th</sup> and 28<sup>th</sup> day.



**Graph 1.** Comparison of the occurred apical microleakage between groups on 7<sup>th</sup> day and 28<sup>th</sup> day (CPM3 – Counts per 3 minutes) (PG = positive control group; NG = negative control group; G1 = experimental group, prior use of calcium hydroxide as medication; G2 = experimental group without prior medication)

The positive control group presented significantly higher radioactivity ( $p < 0.01$ ), compared with other groups. On the other hand, the negative control group presented a significantly lower radioactivity ( $p < 0.005$ ), compared with other groups.

The statistically significant differences between control groups and experimental groups were observed at both phases, on the 7<sup>th</sup> and 28<sup>th</sup> day. Significant differences were not found between the two experimental groups, neither on the 7<sup>th</sup> day ( $p = 0.8$ ) nor on the 28<sup>th</sup> day ( $p = 1$ ) after placement of the MTA apical plug. In all groups statistically significant differences were not found between the two periods (7<sup>th</sup> day and 28<sup>th</sup> day) (Table 1).

**Table 1.** Means and standard deviations of each group on the 7<sup>th</sup> and 28<sup>th</sup> day and p-values related to the differences found between both periods

group	n	7 <sup>th</sup> day		28 <sup>th</sup> day		p-value
		means	standard deviation	mean	standard deviation	
PG	7	0,94	0,46	1,05	0,37	0,9165
NG	7	0,03	0,01	0,04	0,02	0,1797
G1	10	0,21	0,10	0,24	0,16	0,8139
G2	10	0,29	0,13	0,27	0,22	0,4802

The means of the results obtained in both experimental groups (G1 with prior use of calcium hydroxide as medication and G2 – without prior medication) on 7<sup>th</sup> and 28<sup>th</sup> day are presents on Graph 1. Despite the lack of statistically significant differences, as mentioned above, G1 shows less leakage on both days. Nevertheless, G1 presents greater infiltration on the 28<sup>th</sup> day than on the 7<sup>th</sup> day, whereas in G2 the results are inverse.

## Discussion

The present experimental study was designed to evaluate the effect of Ca(OH)<sub>2</sub> on the apical seal with MTA, placed as an apical plug in immature simulated teeth. The significantly higher leakage associated with the positive control group compared to the other groups on 7<sup>th</sup> and 28<sup>th</sup> day shows that, in the absence of the MTA apical plug, the solution containing the <sup>99m</sup>Tc was able to penetrate, and that MTA functions well as filling material, significantly reducing infiltration. In turn, the negative control group showed significantly lower values of infiltration, demonstrating that the two varnish layers were effective in sealing the root surfaces, thus preventing side microleakage. There were no statistically significant differences related to the apical leakage between the group previously treated with Ca(OH)<sub>2</sub> and the group that was not previously medicated. This lack of statistically significant difference was noted both on 7<sup>th</sup> and 28<sup>th</sup> day after placing the White MTA (WMTA) apical plug. These results are in agreement with those obtained in the *in vitro* study of Hachmeister *et al.*<sup>1</sup> (2002) using a microbiological model for detection of apical leakage, who did not find any effect of pretreatment with Ca(OH)<sub>2</sub> in the sealing performance of Gray MTA (GMTA) placed as an apical plug. Despite the lack of statistical significance in the present study, there was found a lower microleakage in the specimens of G1 compared to the G2 at both periods, on the 7<sup>th</sup> day and 28<sup>th</sup> days. A possible reason for this finding could be that calcium hydroxide may form a layer between MTA and dentin, thus blocking the dentinal tubules. Another aim of the present study was to evaluate the sealing ability of MTA apical plug over time. Mileti *et al.*<sup>17</sup> (2002) emphasize the need to determine infiltration at different periods as the sealing ability of a given material can change over time. In the present study, there were not found any statistically significant differences between the two selected periods. On the 28<sup>th</sup> day, a slight increase of infiltration in G1 and a slight decrease of infiltration in G2 were observed, although on both days the previously medicated (G1) presents slightly lower values of infiltration than the group not previously medicated (G2) (Graph 1). Given these results, it is possible raise the question if over a certain time the sealing ability of MTA could improve, possibly due to the fact that the final setting is only completed at 21 days<sup>18</sup>. However, improvement was not observed in the group previously treated with calcium hydroxide, which may be due to the fact that over time dissolution of Ca(OH)<sub>2</sub> may occur, resulting in gaps, leading to higher infiltration values. Thus, the remaining calcium hydroxide may reduce the infiltration only temporarily. Perhaps the selection of the second period should

be after the 28 days to provide more time for the complete dissolution of calcium hydroxide and assess the influence of its use on long term MTA apical sealing. However, because of the lack of statistical significance, the observed differences may also be due to chance or unidentified variables. Several studies were performed to evaluate the influence of residual calcium hydroxide for various filling materials, with different results. Based on these studies, hypotheses have emerged about the possible means of influence of Ca(OH)<sub>2</sub> on obturation cements and MTA. Tsimas and Tsvilis<sup>19</sup> (2001) report that a chemical reaction can occur between the residual Ca(OH)<sub>2</sub> and different filling materials, which may affect their sealing ability. Residual calcium hydroxide can also prevent the entry of filling materials in the dentinal tubules, leading to a lower capacity of sealing and consequent greater infiltration. At the same time, just as the calcium hydroxide can affect the entry in the dentinal tubules of the filling materials it can also affect the penetration of tracers, especially dyes, and may thus result in lower leakage values as found in some studies<sup>14,20-23</sup>. However, this initial improvement of apical seal may be temporary, since the calcium hydroxide can form calcium carbonate by a chemical reaction, which is an absorbable substance, thus resulting in possible cracks in these areas and increased microleakage in the long term<sup>12,14,16</sup>. Further, it is hypothesized that Ca(OH)<sub>2</sub> can be incorporated in the filling materials, thereby decreasing its permeability<sup>21</sup>. Additionally, calcium hydroxide may produce a residual expansion of filling materials and thus increase its sealing ability<sup>20</sup>. It is not known what effect residual Ca(OH)<sub>2</sub> may have on the formation of the hydroxyapatite-type-layer on the surface of MTA, thought to promote an initial chemical and posterior mechanical bond between dentin and MTA<sup>24</sup>. The methodologies used to determine infiltration differ widely between the existing studies, thus limiting the interpretation of the results. Several methods have been used to assess the apical sealing ability of different materials and techniques, by determining apical microleakage. Many infiltration studies use subjective methods, thereby increasing the margin of error. Concerning dye-leakage studies using methylene blue dye, false negative results may have occurred, because this substance may lose its colour in contact with calcium hydroxide<sup>25-26</sup>. The use of the radionuclide <sup>99m</sup>Tc, as a tracer, and the gamma camera for detecting the radiation emitted by the teeth allows obtaining quantitative data and comparing different periods, using the same sample without need to carry out any cutting of teeth. The radioisotope <sup>99m</sup>Tc is an emitter of pure gamma radiation, with low energy (140 KeV) and presents as an advantage its short half-life of 6 hours<sup>27</sup>. After its production, <sup>99m</sup>Tc presents stability over a period of 4-8 hours<sup>28</sup>. The methodology of determining the leakage via detection of radioactivity by gamma camera may be advantageous, allowing a quantitative analysis and comparison between different periods. As for future perspectives, it is noteworthy that several authors, based on several published case reports, where maturogenesis occurred after application of regenerative procedures, advocate a paradigm shift regarding the clinical management of necrotic immature permanent teeth with apical perio-

dontitis, replacing the usual procedure of apexification by regeneration procedures as the main therapeutic option<sup>29,30</sup>. However, despite the emergent potential of pulp regeneration, apexification with MTA will still be indicated in cases where the regenerative endodontic procedures do not result in maturogenesis<sup>2,29</sup>.

## Conclusion

According to the results obtained in the present study, dressing with calcium hydroxide did not affect the apical sealing ability of the MTA plug on the 7<sup>th</sup> and 28<sup>th</sup> day and there were no statistically significant differences of apical leakage between the different selected periods, the 7<sup>th</sup> and 28<sup>th</sup> day after placing the apical barrier with MTA. *In vivo* studies are recommended to obtain data with clinical relevance.

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