Mestrado Integrado em Medicina Dentária

Faculdade de Medicina da Universidade de Coimbra

1290

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Apical sealing ability of two calcium-silicate based cements using a radioactive isotope method: an *in vitro* apexification model

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Coimbra, julho 2021

Apical sealing ability of two calcium-silicate based cements using a radioactive isotope method: an *in vitro* apexification model

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Coimbra, julho 2021

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ABSTRACT

Introduction: Pulp necrosis in immature teeth is an endodontic challenge. In these cases, it has been used apical plugs to promote apexification, with mineral trioxide aggregate (MTA) being the gold-standard material. However, the search for an ideal material is ongoing and new bioceramic cements have been introduced as an alternative.

Aim: The aim of the present study is to evaluate and compare the sealing ability of two calcium-silicate based cements (TotalFill BC RRM Fast Set Putty and White ProRoot MTA) when used as apical plugs in teeth with open apices, trough Nuclear Medicine.

Materials and Methods: Thirty-four single-rooted extracted teeth had its crowns and root tip sectioned to obtain 14mm long root segments to simulate an *in vitro* apexification model. The teeth were prepared using mechanized instrumentation. Two experimental groups of 12 teeth each (MTA group and BC group) and two control groups (positive and negative), each with 5 teeth, were created. On the 4th day after placement of the respective apical plug, the apical portion of the teeth were submerged in a solution of sodium pertechnetate ($^{99m}TcNaO_4$) for 3 hours. The radioactivity was measured using a gamma camera.

Results: The present study showed that none of the root canal-filled teeth was leakage free. Statistical analysis showed a statistically significant difference between the MTA group and the controls ($p<0.05$). The BC group had significant differences regarding the negative control ($p<0.001$) but showed no statistical significance regarding the positive control ($p=0.168$). There was a statistically significant difference ($p=0.009$) between the BC group (7335.8 ± 2755.5) and the MTA group (4059.1 ± 1231.1), where the MTA group showed less infiltration of $^{99m}TcNaO_4$.

Conclusions: Within the limitations of this study, White ProRoot MTA had a significantly better sealing ability than TotalFill BC RRM Fast Set Putty.

Keywords: Apexification; Calcium Silicate; Dental Leakage; Mineral Trioxide Aggregate; Sodium Pertechnetate Tc 99m.

RESUMO

Introdução: A necrose pulpar em dentes imaturos é um desafio endodôntico. Nestes casos, tem-se recorrido à utilização de *plugs* apicais para promover a apexificação, em que o agregado de trióxido mineral (MTA) é o material *gold-standard*. No entanto, a procura por um material ideal ainda decorre e novos cimentos biocerâmicos têm sido introduzidos como alternativa.

Objetivo: O objetivo do presente estudo é avaliar e comparar a capacidade de selagem de dois cimentos de silicato de cálcio (TotalFill BC RRM Fast Set Putty e White ProRoot MTA) quando usados como *plugs* apicais em dentes com ápice aberto, através de Medicina Nuclear.

Materiais e Métodos: Trinta e quatro dentes extraídos, com uma única raiz, foram seccionados na coroa e ápice, obtendo-se segmentos de 14mm de comprimento, de forma a simular um modelo de apexificação *in vitro*. Os dentes foram preparados com instrumentação mecanizada. Dois grupos experimentais com 12 dentes cada (grupo MTA e grupo BC) e dois grupos de controlo (positivo e negativo) com 5 dentes cada, foram estabelecidos. Ao 4º dia após a colocação do respetivo *plug* apical, a porção apical dos dentes foi submersa numa solução de pertecnetato de sódio ($^{99m}TcNaO_4$) por 3 horas. A radioatividade foi medida usando uma gamma câmara.

Resultados: O presente estudo demonstrou que nenhum dos dentes obturados ficou livre de infiltração. A análise estatística demonstrou uma diferença estatisticamente significativa entre o grupo MTA e os controlos ($p<0,05$). O grupo BC teve uma diferença significativa em relação ao controlo negativo ($p<0,001$), mas não demonstrou uma significância estatística em relação ao controlo positivo ($p=0,168$). Houve uma diferença estatisticamente significativa ($p=0,009$) entre o grupo BC (7335,8 ± 2755,5) e o grupo MTA (4059,1 ± 1231,1), em que o grupo MTA teve menor infiltração de $^{99m}TcNaO_4$.

Conclusões: Dentro das limitações deste estudo, o White ProRoot MTA teve uma capacidade de selagem significativamente melhor que o TotalFill BC RRM Fast Set Putty.

Palavras-chave: Agregado de Trióxido Mineral; Apexificação; Infiltração Dentária; Pertecnetato Tc 99m de Sódio; Silicato de Cálcio.

INTRODUCTION

Pulp necrosis in an immature tooth can be caused by trauma, damaging Hertwig's root sheath (HERS)[1] or by delayed diagnosis and treatment of deep carious lesions.[2] The root development of such teeth is interrupted, resulting in an incomplete root formation characterized by unreached final root length, thin root dentinal wall, wide canal space, apical divergence, absence of an apical constriction and an open apex.[3] Such inherent characteristics can be an endodontic challenge.[4] Essentially due to the divergent apical architecture and lack of apical constriction that can cause extrusion of irrigants, medicine and root canal fillings beyond the apex, making the complete debridement and control of the filling nearly impossible, compromising the long-term outcome of the treatment.[4–6]

These challenges require specific approaches such as a chemical disinfection protocol with minimal instrumentation and a softened filling technique.[7],[8] The current treatment options for teeth with open apex are apexification, placing an apical plug or regenerative endodontic treatment.[8]

The traditional approach to handle cases of necrotic teeth with an open apex used to be multiple-visit apexification treatment using calcium hydroxide ($Ca(OH)_2$) as intracanal medicament.[9] Although $Ca(OH)_2$ apexification has high clinical success rates and most of the teeth exhibit a calcified barrier at the apical portion, several limitations led to seek for alternative treatment modalities.[9] The long time for the apical barrier to be formed, the need for multiple visits, the possibility of coronal microleakage during treatment as well as the lower resistance to fracture of the teeth are some of those disadvantages.[9]

The placement of an apical plug is one alternative to calcium hydroxide apexification.[10] Apical plug has been described as the non-surgical compaction of a biocompatible material into the apical end of the root canal that prevents the passage of toxins and bacteria into periradicular tissue.[11],[12] This barrier enables the placement of an appropriate root canal sealant and filling material, whilst reducing the possibility of their extrusion into periapical tissues, preventing overfilling and promoting periapical healing.[7],[12],[13]

Researchers have demonstrated that a proper apical sealing ability is the most important factor for achieving success in endodontic procedures, because it is responsible to prevent bacteria and their byproducts from permeating periapical tissue.[5] Several studies have shown that an apical plug of 3 to 5 mm is sufficient to achieve an adequate seal in apical barrier techniques.[5]

Therefore, an ideal endodontic cement material should seal the pathway between the root canal space and the surrounding tissues, providing resistance to leakage.(14),(15) In addition, it needs to be nontoxic, radiopaque, biocompatible, bacteriostatic, insoluble in tissue fluids, dimensionally stable, easy to prepare and handle and have a proper setting time and biomimetic properties.(14–16) Although no endodontic cement meets all properties of Grossman's ideal, there are many endodontic cements available. They can be categorized according to their main chemical constituents: zinc oxide eugenol, calcium hydroxide, glass ionomer, silicone, resin, and bioceramic-based cements.(17)

Bioceramic-based cements have only been available for use in endodontics for the past thirty years and were designed specifically for medical and dental use.(17) They are biocompatible, non-toxic and chemically stable within the biological environment.(18) A further advantage is their ability to form hydroxyapatite and create a bond between dentin and the material, contributing to the sealing ability of the material.(18),(19) The classification of bioceramics into bioactive or bioinert materials is according to their interaction with the surrounding living tissue.(17) Bioactive materials, such as glass and calcium phosphate, interact with the surrounding tissue to encourage the growth of more durable tissues.(17) Bioinert materials, such as zirconia and alumina, produce an insignificant response from the surrounding tissue, having no biological or physiological effect.(17) Bioactive materials are further classified according to their stability as degradable or nondegradable.(17)

Following the introduction of bioceramic materials, an apical plug with mineral trioxide aggregate (MTA) has become the gold standard for treatment of teeth with open apex.(5),(16),(20) It is used not only for root-end filling but also for procedures like pulp capping, pulpotomy, apexogenesis, apexification, root perforations repair, and root canal filling.(16),(20) MTA is a calcium silicate-based cement with dicalcium silicate, tricalcium silicate, tricalcium aluminate, bismuth oxide and calcium sulfate in its composition.(21–23) However, MTA has some disadvantages, including tooth discoloration, difficult manipulation, prolonged setting time and high cost.(5),(14),(24) A white MTA (WMTA) has gained popularity following reports of coronal discoloration with grey MTA (GMTA), in an attempt to achieve better aesthetic results.(1),(14),(25) The major difference between GMTA and WMTA is in the concentrations of metal oxides content such as aluminium oxide, magnesium oxide and iron oxide.(1),(26)

Given these drawbacks, the search for an ideal apical plug material is ongoing.(24)

Recently, a class of new bioceramic materials have been introduced as an alternative to MTA, which owns many similar and some different characteristics, in an effort to develop the

Grossman's ideal material[18] and with claimed improved handling characteristics.[18],[22],[27] They are composed of tricalcium silicate, dicalcium silicate, zirconium oxide, tantalum pentoxide, calcium phosphate monobasic (BC) and calcium phosphate.[21],[22],[28] It can be presented in vary formats: paste, putty and fast set putty.[22] Studies have shown that it does not induce discoloration in the tooth structure as well.[28]

However, few published studies have compared the sealing ability of these bioceramic cements when used as apical plugs in teeth with open apices.

The aim of the present study is to evaluate and compare the sealing ability of two calcium-silicate based cements (TotalFill Bioceramic Root Repair Material Fast Set Putty and White ProRoot MTA) used as apical plugs in teeth with open apices, trough Nuclear Medicine.

The null hypothesis is that there are no significant differences in the sealing ability of the calcium-silicate based cements White ProRoot MTA and the TotalFill BC RRM Fast Set Putty.

MATERIALS AND METHODS

1. <u>Collection of the sample</u>

For this project, 34 maxillary and mandibular monoradicular premolars that were extracted due to orthodontic or periodontal reasons were used. After that, the periodontal ligament or calculus that might be present were removed with Gracey curettes. The teeth were cleaned with NaOCl at 2.5% and preserved in chloramine T at 4°C until used, to prevent bacterial growth.

The teeth selected had to be straight, with a single and permeable canal and fully formed roots. The exclusion criteria were calcified canals, teeth with obstructions inside the canal, internal or external root resorption, with previous endodontic treatment, fractures or root fissures, caries lesions or restorations from cementoenamel junction (CEJ) level to the apex and without pronounced apical curvature.

To standardize the selection of teeth to a similar internal anatomy and to confirm the existence of a single canal, preoperative radiographs were performed in an orthogonal and proximal projections. The teeth were examined through the optical microscope to assess that there were no fissures or fractures.

2. <u>Endodontic cements</u>

The endodontic cements used were calcium-silicate based TotalFill BC RRM Fast Set Putty (FKG, La Chaux-de-Fonds, Switzerland) and ProRoot WMTA (Dentsply Maillefer, Ballaigues, Switzerland). The manufacturer, composition and lot no. are presented in table 1.

Table 1. *Manufacturer, c*omposition, and lot no. of the endodontic cements used.

Material	Manufacturer	Composition	Lot no.
TotalFill BC RRM Fast Set Putty®	FKG, La Chaux-de-Fonds, Switzerland	Tricalcium silicate, dicalcium silicate, zirconium oxide, tantalum pentoxide, calcium sulfate (anhydrous) and calcium phosphate monobasic	2003FSPS
ProRoot WMTA®	Dentsply Maillefer, Ballaigues, Switzerland	Dicalcium silicate, tricalcium silicate, tricalcium aluminate, bismuth oxide, calcium sulfate, aluminium oxide, magnesium oxide and iron oxide	0000172755

3. Preparation

To standardize the sample, the crowns were sectioned at the CEJ level with a cylindrical diamond drill, mounted on a high-speed handpiece, perpendicular to the long root axis, in order to obtain root segments with 17mm long. In the teeth where the canal was not exposed, an access was made with a diamond spherical drill mounted on a high-speed handpiece.

The apical portion of the root (3mm) was sectioned with a cylindrical diamond drill mounted on a high-speed handpiece, to simulate an immature tooth. Then, the coronal and root surfaces were smoothened with a *Carburundum* disc. A final length of 14mm was obtained for the specimens. (Fig.1)

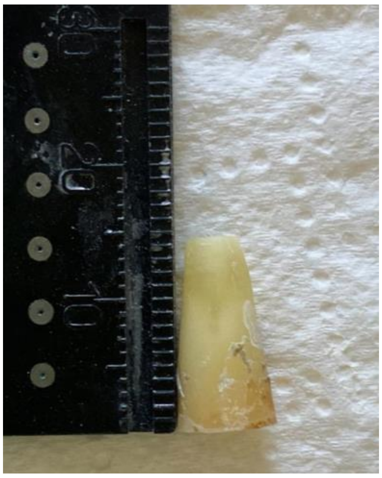

Figure 1. Example of the exterior of the samples.

After that, an exploration and permeabilization of the root canal was made with a 25mm manual K file, ISO size #10 (Dentsply Maillefer, Ballaigues, Switzerland). The working length was established at 14mm by inserting a K file ISO #10 (Dentsply Maillefer, Ballaigues, Switzerland) until it was visible in the apical foramen. The biomechanical orthograde preparation was firstly made by manual instrumentation with a manual K file ISO #10, #15 and #20 (Dentsply Maillefer, Ballaigues, Switzerland) and then by mechanical instrumentation with 25mm Protaper Next (Dentsply Maillefer, Ballaigues, Switzerland,) in X1 (17.04), X2 (25.06) and X3 (30.07) sequence at 14mm, with constant speed of 250 rpm and torque control of 1.2N/cm, using the electric motor X-SMART™ (Dentsply Maillefer, Ballaigues, Switzerland). The irrigation was made with 2mL of NaOCl (sodium hypochlorite) at 2.5% with an irrigation needle 27G, with close end and lateral opening, between files.

To simulate teeth with open apex (Fig.2), a divergent retrograde preparation was created by ProFile Orifice Shaper® (Dentsply Maillefer, Ballaigues, Switzerland) at OS#1 (06/20), OS#2 (06/25) and OS#3 (06/30) sequence introduced at the apical 10mm, through an electric motor X-SMART™ (Dentsply Maillefer, Ballaigues, Switzerland), with constant speed of 250 rpm and torque control of 1.2N/cm. The irrigation procedure was the same as in the orthograde preparation.

Figure 2. Periapical radiograph to demonstrate the simulation of an open apex created with a retrograde instrumentation.

A final irrigation was performed with 1mL of ethylenediaminetetraacetic acid (EDTA) (Magnum Dental AS, Aardla, Estonia) at 17% for 1 minute (to dissolve the inorganic component of the smear layer), 2mL of NaOCl at 2.5% (to remove the organic component of the smear layer) and 2mL of NaCl at 0.9%. The canal was dried with paper points and the integrity of the roots were checked with an optical microscope (Leica® M320, Wetzlar, Germany).

4. **Groups**

Following the canal preparation, a total of 34 root segments were randomly divided into 4 groups.

Two experimental groups with 12 teeth each:

- ProRoot White MTA group (MTAG, n=12);
- TotalFill BC RRM Fast Set Putty group (BCG, n=12).

Two control groups with 5 teeth each:

- Negative control group (NG, n=5);
- Positive control group (PG, n=5).

5. **Obturation**

A 4mm apical plug was placed in the 3 groups, except in the PG group, where the teeth were left without apical obturation material. In the NG and the MTAG group, a 4mm ProRoot White MTA apical plug was placed. In the BCG group a 4mm TotalFill BC RRM Fast Set Putty apical plug was placed.

The WMTA was handled according to the manufacturers' instructions. Regarding to the BC RRM Fast Set Putty, as it is a pre-mixed material ready to use, no preparation of the material was necessary. The respective materials were applied with a MAP System (Micro Apical Placement System) (Dentsply Maillefer, Ballaigues, Switzerland) with a diameter of 1.1mm. The cement was compacted with Schilder Pluggers (Dentsply Maillefer, Ballaigues, Switzerland) with a back packing technique from 14mm to 10mm length, creating a 4mm thick apical plug (Fig.3 and 4). The placement of the endodontic cement was performed under an optical microscope (Leica® M320, Wetzlar, Germany).

Figure 3. Coronal view of the 4mm apical plug.

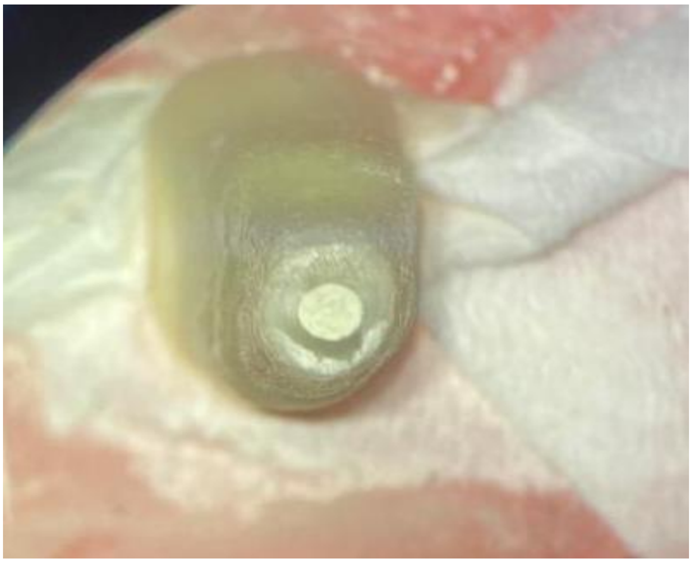

Figure 4. Apical view of the 4mm apical plug.

Each group was placed in its recipient, containing florist sponges previously soaked in chloramine T, with the intent to simulate the soft periapical tissues. To create an atmosphere comparable to *in vivo*, the specimens were storage at room temperature in an environment with 100% relative humidity for 4 days, allowing the complete set of the cements.

6. Infiltration

In the experimental and positive control groups, two layers of nail varnish were applied to the outer surface of each root, except at the last 1mm root tip. In the negative control group, the entire root segment, including the root tip was sealed with varnish.

At the 4th day after the placement of the cements, the apical region of the specimen was submerged in a 50µL solution (8mCi/mL) of sodium pertechnetate ($^{99m}TcNaO_4$) placed in radioimmunoassay tubes for 3 hours. After this period, the apex of the roots was removed from the solution, washed in running water for 30 seconds and the varnish was scraped with a scalpel blade no. 13, to remove the radioisotope vestiges present on the outer surface of the root segments.

To evaluate the apical microleakage, the radioactivity emitted by the specimens was measured through a gamma camera (GE Millennium MG, Milwaukee, United States) controlled by an acquisition computer (GenieAcq). For each tooth, a static image was acquired for two minutes at a 512 × 512 matrix size and zoom of 1.33. Regions of interest (ROIs) in each image were drawn over each tooth, to obtain the total counts, maximums and average. The total counts obtained in each image were used to define the degree of infiltration.

7. Statistical analysis

The statistical analysis was performed by ANOVA 1-factor with a Games-Howell post-hoc test, using IBM SPSS statistic software (version 27). The statistical significance was set at 0.05 ($p<0.05$).

RESULTS

The means and standard deviations (SD) of counts per minute (CPM), for the total of 2 minutes exposure of the specimens, are given in Figure 5.

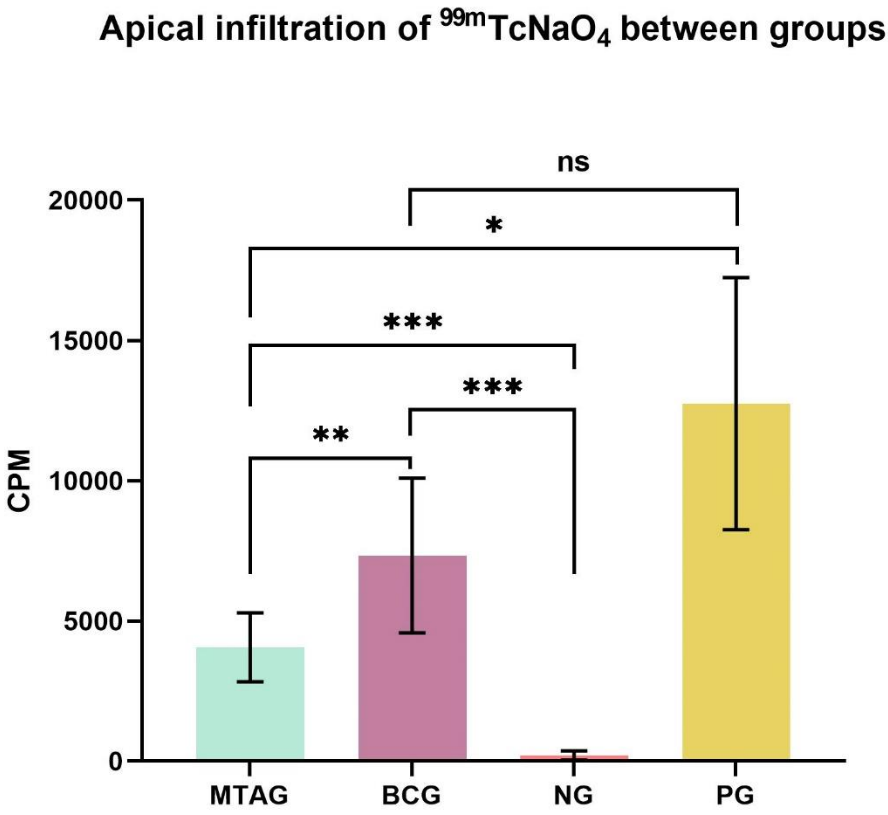

Figure 5. Visual representation of the mean values, standard deviation of the total counts and level of significance: *p<0.05; **p<0.01; ***p<0.001; ns – no significance

Statistical analysis showed a statistically significant increased ($p<0.001$) of the total counts of MTAG (4059.1 ± 1231.1) when compared with the NG (202.2 ± 161.5). Regarding the PG (12746.0 ± 4497.7), the MTAG (4059.1 ± 1231.1) showed a significant decrease of the total counts ($p=0.039$).

The BCG (7335.8 ± 2755.5) showed a statistically significant increase of the total counts ($p<0.001$) when compared with the NG (202.2 ± 161.5). No significant difference ($p=0.168$) was found between BCG (7335.8 ± 2755.5) and the PG (12746.0 ± 4497.7).

Regarding the two experimental groups, BCG (7335.8 ± 2755.5) showed a statistically increased of the total counts ($p=0.009$) comparing with MTAG (4059.1 ± 1231.1).

DISCUSSION

Immature permanent teeth with open apices may develop pulp necrosis as a result of trauma or caries with pulp involvement.[2] A good sealing ability is essential to the longevity of these teeth, because it prevents bacterial leakage.[5],[6] As it is known, the presence of microorganisms in the root canal is the main etiological factor of pulp diseases or endodontic unsuccess, so in order to obtain a good sealing ability, the choice of the cement is key.[29] Due to its physical, chemical and biological properties, calcium-silicate based cements seem to be an acceptable fit.[5],[18]

The present experimental study was designed to evaluate the apical sealing ability of two calcium-silicate based cements, placed as an apical plug in immature simulated teeth, using a solution of sodium pertechnetate ($^{99m}TcNaO_4$). Technetium is an artificial element obtained by the radioactive decay of molybdenum and decays with a half-life of 6 hours by isometric transition and emission of 140.5 keV of gamma radiation.[30] Nevertheless, apical sealing ability can be studied through various methods such as the use of dyes; glucose penetration; bacterial or toxins infiltration; radioactive isotopes; protein microleakage; electrochemical microleakage or scanning electron microscopy.[31],[32]

The most common methods applied to evaluate the sealing ability in recent studies are dye and bacterial leakage. Nonetheless, dye penetration using methylene blue has some limitations such as the necessity to destroy the specimens, the subjective evaluation of the results that request an expertise operator and the semiquantitative nature of measuring microleakage.[33] The bacterial infiltration method, although it might be more clinical and biologically relevant when compared to dye penetration, can only be applied in cements that have antimicrobial activity to the type of bacteria employed and usually have a qualitative rather than a quantitative nature.[32] The use of methods using radioactive isotopes like $^{99m}TcNaO_4$ have a quantitative and nondestructive nature enabling measurement of microleakage from the same specimens at intervals over extended periods, without the need to destroyed the sample, contrasting with the two methods mentioned.[34] It also allows detection per minute of the amount of leakage and has the advantage over dyes as tracers, for their presence can be readily detected in very small concentrations.[35],[36]

The significantly higher leakage associated with the positive control group compared to the other groups in the present study, shows that in the absence of an apical plug, the solution containing the $^{99m}TcNaO_4$ was able to penetrate.[34] On the other hand, 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 infiltration and confirming the method used in this study.[34]

There are doubts about the value of *in vitro* leakage studies in terms of clinical significance and limitations of the results and lack of technique standardization. Based on available evidence on the study of Jafari *et al.*, it appears that despite potential errors on leakage studies they are very valuable.[32] Furthermore, sealing ability is responsible to prevent bacteria and their byproducts from permeating periapical tissue, as already mentioned.[5] Therefore, this characteristic remains a priority when evaluating and comparing new apical plug materials before they are used in the clinic.[21],[32]

The standardization of *in vitro* conditions is an important step for controlling possible biases and optimizing the statistical analysis.[37] In this sense, it was important in this research to try to standardize the internal anatomy of the sample[37] by using human extracted teeth with the inclusion criteria mentioned above. The restriction towards the characteristics of the sample performed a conditioning factor of the number of teeth included. The open apex was created using a previously described retrograde instrumentation technique by Hachmeister *et. al.*,[4] with modifications already stated. Due to the importance of successful debridement of root canals during cleaning and shaping procedures, the irrigation process with chemical irrigants was firstly made with EDTA.[35] Still, it is known that EDTA alone does not completely remove the smear layer, and that the best results are achieved with 1 minute irrigation of EDTA combined with a sodium hypochlorite solution.[38] A final irrigation step was made with saline since using solutions such as EDTA and sodium hypochlorite leaves crystals on the canal walls.[38]

A thickness of 4mm of the cement was placed as an apical plug in this study, since many leakage studies have revealed that a 4mm thickness of an apical plug material provides a significantly good seal.[39–41] In a previous study, Lertmalapong *et al.*, concluded that TotalFill BC RRM putty and ProRoo MTA at a thickness of 4mm, exhibited the best sealing ability and marginal adaptation.[5] The possible reason given was that a greater mass of material results in greater expansion, reducing the gap area.[42] This expansion is offered by the calcium silicate in their composition, which expands by 0.2% to 0.6% of its initial volume.[42] This expansion greatly contributes to its sealing capacity.[42]

Materials based on tricalcium silicate such as WMTA and BC RRM are hydraulic because they have the ability to set in the presence of moisture.[43] As so, the obturated teeth were kept in floral foam to replicate *in vivo* periapical ligament moisture.[44] As the study of

Caronna *et al.* shows, the bioceramic materials can absorb sufficient moisture from a periapical environment to complete the setting reaction without a moistened cotton pellet being required.(23)

The bioceramic material used (BC RRM) was a fast set putty known in Europe as TotalFill by FKG but in USA, it is known as EndoSequence by Brasseler.(45),(46) Consequently, Endosequence and TotalFill BC RRM putty have the same composition.(21),(22),(28),(47) The premixed syringeable ready-to-use formulation provides the clinician a homogeneous and consistent material.(45–47) Its fast-setting working time (20 to 30 minutes) is due to the significantly smaller particle size, which accelerates the setting reaction.(28) Also, because the material is premixed with nonaqueous but water-miscible carriers, it will not set during storage, only on exposure to an aqueous environment, eliminating the potential of heterogenous consistency during on-site mixing, as it might happen in handling of WMTA.(48),(49)

There are few studies comparing the sealing ability of these two materials, and usually it is used dye penetration of methylene blue or bacterial leakage of *Enterococcus faecalis*. At the best of our knowledge, there is no other study comparing the two materials using radioactive isotopes. Moreover, studies about the sealing ability of BC RRM compared to MTA have shown conflicting results.(21),(22),(27),(50),(51)

Under the conditions of this study, there was a statistically significant difference between WMTA and TotalFill BCRRM Fast Set Putty (p=0.009), where the BCRRM had the highest leakage. This was in agreement with a previous study on Endosequence BC RRM by Hirschberg *et al.*(51) However, other studies reported no significant difference in the two materials,(21),(22),(50) although, in the Nair *et al.* study, the BCRRM had more percentage of leakage.(21) On the other hand, Jeevani *et al.* showed that BC RRM had less leakage than MTA.(50)

The discrepancies may be related to different methodologies. Matloff *et al.* did not report any correlation between dye penetration studies and radioisotope techniques.(32) Similarly, a study by Barthell *et al.* showed no correlation between dye penetration and bacterial leakage techniques.(32) The majority of studies have shown no correlation between the different methods.(32)

The probable cause for significantly more leakage of TotalFill BC RRM Fast Set Putty compared to the WMTA in this study could be due to the fast-working time and putty consistency that was considered hard to introduce and manipulate into the root canal. Some affinity for the metal instruments used (MAP System and Schilder Pluggers) was also recorded.

This might led to a compromised marginal adaptation that preceded the poor sealing ability registered.[5] Other probable cause could be the presence of voids.[27] In the study of Alsubait *et al.*, the bond failure observed in BC RRM Fast Set Putty was predominantly within the material itself.[46]

The evaluation of apical infiltration after four days of placing the apical plug was chosen to allow the materials an adequate time for setting. Although setting time could be another factor for the results obtained, according to the manufacturer, working time of BCRRM Fast Set Putty is about 30 minutes and setting time is about 4 hours under normal conditions, for BCRRM and MTA.[21] However, BCRRM may take up to 12 hours under extremely dry conditions.[21] In the study of Guo *et al.* the initial and final setting time of ERRM Putty was significantly longer than WMTA, but at the 4th day of setting, reached the same level of microhardness as WMTA.[48] For this reason, when the infiltration was performed at the 4th day, the two materials were in equal stages of setting.

The sealing ability of MTA has been widely investigated and documented in the literature. Within the results of our study, there was some apical infiltration of $^{99m}TcNaO_4$ in the MTA group. Nevertheless, they were significantly lower than the positive control, thus corroborating the fact that has good sealing ability. This is in agreement with previous studies that state that the incidence of leakage in MTA apical plug can be explained by the presence of failures within the cement, at the cement-dentin interface and/or by the presence of pores interconnected to MTA.[52]

Also, the presence of artifacts such as cracks or surface debris nonvisible through the microscope and variations in the angulation of the root or apex may accentuate the appearance of gap defects[19] that led to infiltration of the radioisotope in both experimental groups.

However, the results of *in vitro* studies cannot be directly anticipated to the clinical situation.[22] Although this study suggests that WMTA has better sealing ability than TotalFill BC RRM Fast Set Putty, more *in vitro* studies, with a significantly larger sample size and measuring the infiltration at intervals over extended periods (at 7th and 28th days, for example) should be made to further investigate the behavior of these materials when exposed to *in vivo* conditions. Nevertheless, the search for an ideal apical plug material is still on pursuit. Until then, choosing the gold-standard MTA seems like a very thoughtful and literature-based decision.

CONCLUSION

Within the limitations of this *in vitro* study, it can be concluded that for treatment of teeth with open apices requiring orthograde delivery of an apical plug, White ProRoot MTA has a sealing ability significantly better than TotalFill BC RRM Fast Set Putty.

There was a statistically significant difference in the sealing ability of the calcium-silicate based cements White ProRoot MTA and the TotalFill BC RRM Fast Set Putty, thus rejecting the null hypothesis.

ACKNOWLEDGMENTS

This study combines the end of a difficult but fulfilling journey of the past five years.

My first few words are for my advisor, PhD Manuel Marques Ferreira. I would like to express my gratitude for his professional guidance, knowledge and kindness through the development of this project.

A very special thanks to my co-advisor, Doctor Catarina Craveiro, for always be available to clarify my doubts, helping me throughout the entire study and for being a friendly and reassuring voice.

I would also like to express my gratitude to PhD Margarida Abrantes, for her specialized supervision, promptitude and assistance in the Nuclear Medicine procedures and statistical analysis.

My grateful thanks are also extended to FKG for offering the material for my research project. Without the support of this company, this *in vitro* study would not be possible.

I would like to thank to Alda Neves and Sónia Correia, for their availability and patience in the pre-clinic.

To my colleagues at the clinic and group of friends, I will always cherish the moments throughout our academic path. Without it, this would not be so rewarding and fun.

To Tiago, there are no words to thank the support, patient, and caring words and for always being by my side in the pursuit of being a better person.

To my family, a very special thanks, for believing in me and helping me throughout this entire journey.

REFERENCES

1. Moore A, Howley MF, O'Connell AC. Treatment of open apex teeth using two types of white mineral trioxide aggregate after initial dressing with calcium hydroxide in children. Dent Traumatol. 2011;27(3):166–73.

2. Nicoloso GF, Pötter IG, Rocha R de O, Montagner F, Casagrande L. A comparative evaluation of endodontic treatments for immature necrotic permanent teeth based on clinical and radiographic outcomes: a systematic review and meta-analysis. Int J Paediatr Dent. 2017;27(3):217–27.

3. Waterhouse P, Whitworth J, Camp J, Fuks A. Pediatric endodontics: endodontic treatment for the primary and young permanent dentition. In: Hargreaves KM, Cohen S Cohen´s pathways of the pulp. 10th ed. Saint Louis: Mosby Elsevier; 2011.

4. Hachmeister DR, Schindler WG, Walker WA, Thomas DD. The sealing ability and retention characteristics of mineral trioxide aggregate in a model of apexification. J Endod. 2002;28(5):386–90.

5. Lertmalapong P, Jantarat J, Srisatjaluk RL, Komoltri C. Bacterial leakage and marginal adaptation of various bioceramics as apical plug in open apex model. J Investig Clin Dent. 2019;10(1):e12371.

6. Ørstavik D, Qvist V, Stoltze K. A multivariate analysis of the outcome of endodontic treatment. Eur J Oral Sci. 2004;112(3):224–30.

7. Trope M. Treatment of the Immature Tooth with a Non-Vital Pulp and Apical Periodontitis. Dent Clin North Am. 2010;54(2):313–24. Available from: http://dx.doi.org /10.1016/j.cden.2009.12.006

8. Hargreaves KM, Diogenes A, Teixeira FB. Treatment options: Biological basis of regenerative endodontic procedures. J Endod. 2013;39(3 SUPPL.):S30–43. Available from: http://dx.doi.org/10.1016/j.joen.2012.11.025

9. Agrafioti A, Giannakoulas DG, Filippatos CG, Kontakiotis EG. Analysis of clinical studies related to apexification techniques. Eur J Paediatr Dent. 2017;18(4):273–84.

10. Ayatollahi F, Zarebidoki F, Razavi SH, Tabrizizadeh M, Ayatollahi R, Heydarigujani M. Comparison of Microleakage of CEM Cement Apical Plug in Different Powder/ Liquid Ratio in Immature Teeth Using Fluid Filtration Technique. J Dent. 2019;20(1):37–41.

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

12. Mackie IC. UK National Clinical Guidelines in Paediatric Dentistry. Management and root canal treatment of non-vital immature permanent incisor teeth. Faculty of Dental Surgery, Royal College of Surgeons. Int J Paediatr Dent. 1998 Dec;8(4):289–93.

13. Masmoudi F, Bourmeche I, Sebai A, Baccouche Z, Maatouk F. Root lengthening with apical closure in two maxillary immature permanent central incisors after placement of mineral trioxide aggregate (MTA) as an apical plug. Eur Arch Paediatr Dent [Internet]. 2018;19(1):65–71. Available from: https://doi.org/10.1007/s40368-017-0323-7

14. Parirokh M, Torabinejad M. Mineral Trioxide Aggregate: A Comprehensive Literature Review-Part III: Clinical Applications, Drawbacks, and Mechanism of Action. J Endod. 2010;36(3):400–13. Available from: http://dx.doi.org/10.1016/j.joen.2009.09.009

15. Gartner AH, Dorn SO. Advances in endodontic surgery. Dent Clin North Am. 1992 Apr;36(2):357–78.

16. Kadić S, Baraba A, Miletić I, Ionescu A, Brambilla E, Ivanišević Malčić A, et al. Push-out bond strength of three different calcium silicate-based root-end filling materials after ultrasonic retrograde cavity preparation. Clin Oral Investig. 2018;22(3):1559–65.

17. Al-Haddad A, Aziz ZACA. Bioceramic-Based Root Canal Sealers: A Review. Int J Biomater. 2016.

18. Wang Z. Bioceramic materials in endodontics. Endod Top. 2015;32(1):3–30. Available from: https://doi.org/10.1111/etp.12075

19. Tran D, He J, Glickman GN, Woodmansey KF. Comparative analysis of calcium silicate-based root filling materials using an open apex model. J Endod. 2016;42(4):654–8. Available from: http://dx.doi.org/10.1016/j.joen.2016.01.015

20. Parirokh M, Torabinejad M. Mineral Trioxide Aggregate: A Comprehensive Literature Review-Part I: Chemical, Physical, and Antibacterial Properties. J Endod. 2010;36(1):16–27. Available from: http://dx.doi.org/10.1016/j.joen.2009.09.006

21. Nair U, Ghattas S, Saber M, Natera M, Walker C, Pileggi R. A comparative evaluation of the sealing ability of 2 root-end filling materials: An in vitro leakage study using Enterococcus faecalis. Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology.

2011;112(2):e74–7. Available from: http://dx.doi.org/10.1016/j.tripleo.2011.01.030

22. Antunes HS, Gominho LF, Andrade-Junior C V., Dessaune-Neto N, Alves FRF, Rôças IN, et al. Sealing ability of two root-end filling materials in a bacterial nutrient leakage model. Int Endod J. 2016;49(10):960–5.

23. Caronna V, Himel V, Yu Q, Zhang JF, Sabey K. Comparison of the surface hardness among 3 materials used in an experimental apexification model under moist and dry environments. J Endod. 2014;40(7):986–9.

24. Memiş Özgül B, Bezgin T, Şahin C, Sari Ş. Resistance to leakage of various thicknesses of apical plugs of Bioaggregate using liquid filtration model. Dent Traumatol. 2015;31(3):250–4.

25. Sarris S, Tahmassebi JF, Duggal MS, Cross IA. A clinical evaluation of mineral trioxide aggregate for root-end closure of non-vital immature permanent incisors in children-a pilot study. Dent Traumatol. 2008;24(1):79–85.

26. Asgary S, Parirokh M, Eghbal MJ, Brink F. Chemical differences between white and gray mineral trioxide aggregate. J Endod. 2005;31(2):101–3.

27. Lagisetti AK, Hegde P, Hegde MN. Evaluation of bioceramics and zirconia-reinforced glass ionomer cement in repair of furcation perforations: An in vitro study. J Conserv Dent. 2018;21(2):184–9.

28. Alsubait S, Al-Haidar S, Al-Sharyan N. A Comparison of the Discoloration Potential for EndoSequence Bioceramic Root Repair Material Fast Set Putty and ProRoot MTA in Human Teeth: An In Vitro Study. J Esthet Restor Dent. 2017;29(1):59–67.

29. Siqueira JFJ. Aetiology of root canal treatment failure: why well-treated teeth can fail. Int Endod J. 2001 Jan;34(1):1–10.

30. Mazzi U. Technetium-99m pharmaceuticals: preparation and quality control in nuclear medicine. In: Zolle I, editor. 1st ed. Berlin: Springer; 2007. p. 95.

31. Gogna R, Jagadis S, Shashikal K. A comparative in vitro study of microleakage by a radioactive isotope and compressive strength of three nanofilled composite resin restorations. J Conserv Dent. 2011 Apr;14(2):128–31.

32. Jafari F, Rahimi S, Shahi S, Jafari S. Endodontic microleakage studies: Correlation

among different methods, clinical relevance, and potential laboratory errors. Minerva Stomatol. 2017;66(4):169–77.

33. Camps J, Pashley D. Reliability of the dye penetration studies. J Endod. 2003 Sep;29(9):592–4.

34. Afonso T, Pega MLS de AM, Michelotto AL da C, Abrantes AMC, Oliveiros B, Carrilho EVP, et al. Effect of calcium hydroxide as intracanal medication on the apical sealing ability of mineral trioxide aggregate (MTA): an in vitro apexification model TT - Efeito da medicação intracanal de hidróxido de cálcio na capacidade seladora do mineral trióxido . J Heal Sci Inst. 2012;30(4). Available from: http://www.unip.br/comunicacao/publicacoes/ics/edicoes/2012/04_out-dez/V30_n4_2012_p318a322.pdf

35. Ferreira MM. Quantitative scintigraphic analysis of the apical seal in Thermafil/Topseal and RealSeal 1/Realseal filled root canals. World J Stomatol. 2013;2(2):30.

36. Keshava Prasad B, Sudhakaran S. Radioactive Isotope Evaluation of Coronal Leakage after Endodontic Treatment in Teeth Restored with Three Different Intracoronal Restorative Materials: An in vitro Study. World J Dent. 2011;2(1):35–8.

37. Ribeiras I, Vasconcelos I, Ramos M, Lopes M, Ginjeira A. Estudo comparativo da adaptação marginal de 2 cimentos endodônticos. Rev Port Estomatol Med Dent e Cir Maxilofac. 2015;56(3):173–81. Available from: http://dx.doi.org/10.1016/j.rpemd.2015.06.001

38. Dotto SR, Travassos RMC, de Oliveira EPM, Machado ME de L, Martins JL. Evaluation of ethylenediaminetetraacetic acid (EDTA) solution and gel for smear layer removal. Aust Endod J. 2007;33(2):62—65. Available from: https://doi.org/10.1111/j.1747-4477.2007.00048.x

39. Valois CRA, Costa EDJ. 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 Jan;97(1):108–11.

40. 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 Feb;31(2):117–9.

41. Rahimi S, Shahi S, Lotfi M, Yavari HR, Charehjoo ME. Comparison of microleakage

with three different thicknesses of mineral trioxide aggregate as root-end filling material. J Oral Sci. 2008 Sep;50(3):273–7.

42. Prati C, Gandolfi MG. Calcium silicate bioactive cements: Biological perspectives and clinical applications. Dent Mater. 2015;31(4):351–70. Available from: http://dx.doi.org/10.1016/j.dental.2015.01.004

43. Moinzadeh AT, Aznar Portoles C, Schembri Wismayer P, Camilleri J. Bioactivity potential of endo sequence BC RRM putty. J Endod. 2016;42(4):615–21. Available from: http://dx.doi.org/10.1016/j.joen.2015.12.004

44. Walsh RM, Woodmansey KF, Glickman GN, He J. Evaluation of Compressive Strength of Hydraulic Silicate-based Root-end Filling Materials. J Endod. 2014;40(7):969–72. Available from: http://dx.doi.org/10.1016/j.joen.2013.11.018

45. Zamparini F, Siboni F, Prati C, Taddei P, Gandolfi MG. Properties of calcium silicate-monobasic calcium phosphate materials for endodontics containing tantalum pentoxide and zirconium oxide. Clin Oral Investig. 2019;23(1):445–57.

46. Alsubait S, Alsaad N, Alahmari S, Alfaraj F, Alfawaz H, Alqedairi A. The effect of intracanal medicaments used in Endodontics on the dislocation resistance of two calcium silicate-based filling materials. BMC Oral Health. 2020;20(1):1–7.

47. Shokouhinejad N, Nekoofar MH, Razmi H, Sajadi S, Davies TE, Saghiri MA, et al. Bioactivity of EndoSequence Root Repair Material and Bioaggregate. Int Endod J. 2012;45(12):1127–34.

48. Guo YJ, Du TF, Li HB, Shen Y, Mobuchon C, Hieawy A, et al. Physical properties and hydration behavior of a fast-setting bioceramic endodontic material. BMC Oral Health. 2016;16(1):1–6. Available from: http://dx.doi.org/10.1186/s12903-016-0184-1

49. Xu HHK, Carey LE, Simon CG, Takagi S, Chow LC. Premixed calcium phosphate cements: Synthesis, physical properties, and cell cytotoxicity. Dent Mater. 2007;23(4):433–41.

50. Jeevani E, Jayaprakash T, Bolla N, Vemuri S, Sunil CR, Kalluru RS. Evaluation of sealing ability of MM-MTA, Endosequence, and biodentine as furcation repair materials: UV spectrophotometric analysis. J Conserv Dent. 2014;17(4):340–3.

51. Hirschberg CS, Patel NS, Patel LM, Kadouri DE, Hartwell GR. Comparison of sealing

ability of MTA and EndoSequence Bioceramic Root Repair Material: a bacterial leakage study. Quintessence Int. 2013 May;44(5):e157-62.

52. Cechella B, de Almeida J, Kuntze M, Felippe W. Analysis of sealing ability of endodontic cements apical plugs. J Clin Exp Dent. 2018;10(2):e146–50.