



COMPARISON OF RESULTS FROM TWO DIFFERENT LEAKAGE MODELS TO EVALUATE THE APICAL SEALING OF ENDODONTIC MATERIALS

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ABSTRACT

Introduction: The methods used to evaluate the apical sealing of root canal filling materials have some limitations and great variability of results can be observed. **Aim:** The aim of this study was to compare, in an ex vivo apexification model, the results from bacterial and glucose leakage tests, which were applied in mineral trioxide aggregate (MTA) apical plugs. **Materials and Methods:** Sixty root segments (12mm) were randomly divided into 2 experimental groups (n=30): G1) MTA; G2) MTA + phosphate-buffered saline intracanal. Half of the specimens in each group were submitted to bacterial leakage test with *E. faecalis* for 70 days. The other half was submitted to the glucose leakage test under pressure (103KPa) for 60 min. The results from the two tests were compared based on the number of specimens presenting leakage. Data were analyzed by Fisher's test ($p < 0.05$). **Results:** There was no significant difference between tests for both groups analyzed ($p > 0.05$). **Conclusion:** The results of the present ex vivo study demonstrated that there was no difference between glucose and bacteria leakage evaluation methods, within the parameters of the present study and regardless limitations.

KEYWORDS: Dental Leakage. Enterococcus faecalis. Glucose.
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INTRODUCTION

Many ex vivo methods have been used to evaluate the apical sealing of filling materials, such as bacterial¹ and glucose leakage tests^{2,3}, due to

their clinical relevance. The glucose leakage test has a high degree of specificity and sensitivity⁴ and is useful for quantitative evaluation of leakage occurring through the apical foramen^{2,3}. Nonetheless, it may be

questioned whether glucose leakage test with pressure application can be considered representative for determination of the penetration of fluids or bacteria within the root canal. Conversely, from a clinical perspective,

leakage models with bacteria as markers have the advantage of using the etiologic agent of apical periodontitis⁵ and may provide a precise indicator of sealing ability⁶. However, some limitations are evident, such as the difficulty to determine the quantity of bacteria that reach the apical foramen, since it is possible that a single viable microorganism has reached the lower chamber and then multiplied and indicated leakage⁷.

Based on the limitations and controversies, the issue of microleakage has been intensively discussed^{8,9}. The high variety of evaluation methodologies and the variability of results¹⁰ are among the shortcomings⁸. Therefore, it has been encouraged to compare the various methods for analysis of sealing ability, attempting to reduce the number of comparative techniques used⁸.

Since there is no ideal and reliable leakage model able to reproduce a clinical situation, it seems relevant to compare different tests, which were considered representative for determination of leakage until short ago. The choice for a more sensitive method will permit analysis of the effectiveness of sealing provided by a specific material.

Therefore, the present study compared, *ex vivo*, the results from two different leakage evaluation methods, namely glucose and bacterial penetration, along the MTA apical plug.

MATERIALS AND METHODS

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

The procedures were performed as described by Reyes-Carmona et al.¹¹. 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 no. 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 no. 6 Gates-Glidden drill (\pm 1.50-mm diameter). The final canal rinse was performed with 17% EDTA followed by 1% NaOCl.

Apexification procedures

Sixty root sections were randomly divided into 2 experimental groups (n = 30). Then, the apical cavities were filled, and the root canals dressed as described in Table 1. MTA cement was mixed following the manufacturer's recommendations. The cement mixture was introduced 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 from all root segments to ensure void-free MTA placement and plug thickness.

In group 1, according to the manufacturer's recommendations, a cotton pellet moistened with distilled water was placed in the cervical region of each root segment, which was replaced by a dry pellet after 24 h. In group 2, in order to favor the biomineralization process¹² the remaining canal space was filled with phosphate-buffered saline (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). Thereafter, the root segments were introduced in plastic vials containing floral foam moistened with 20 mL PBS and stored for 2 months at 37°C.

The root segments of each group were randomly divided according to the leakage test performed.

Assembled double chamber and glucose leakage measuring

Thirty root segments (15 per group) were fixed in a device designed to test glucose leakage (adapted from Leal et al.²). The cervical portion of each root segment was fastened in a 2-mL Eppendorf tube with the apical 7 mm protruding through the end. The upper portion of the Eppendorf tube was connected to a screw device through which 0.75 mL of 1 mol L⁻¹ of glucose solution was injected. The Eppendorf tube was attached to a bucket containing 0.75 mL of deionized water, so that the apical 3 mm of the root were immersed in the water. Low-viscosity cyanoacrylate adhesive (Araldite, Brascola, Joinville, SC, Brazil) was used to seal all interfaces and connections.

For the positive control group (n = 2), root segments without apical plug were used. Two teeth with intact crowns to which two layers of nail varnish were applied over the root surface were used as negative control group (n = 2).

A pressure of 103 KPa (15 psi) was created by a compressed air pump (Inalar Compact, NS Indústria de Aparelhos Médicos, São Paulo, SP, Brazil), which was connected to a

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

Groups	Apical plug	Intracanal dressing
1	MTA*	Moistened cotton pellet
2	MTA*	PBS

*MTA Branco - Angelus Soluções Odontológicas, Londrina, PR, Brazil

system constituted by a manometer, a valve to control the pressure and a cannula in which the screw device, connected to the Eppendorf tube, was fixed. The glucose solution was forced into the tube for 60 min. A system was developed to run six root segments simultaneously.

A 10- μ L aliquot of solution contained in the bucket was drawn using a micropipette, and traces of glucose were identified using a glucose kit (Glicose Pap Liquiform, Labtest Diagnóstica, Lagoa Santa, MG, Brazil).

Each aliquot was analyzed using a UV/VSI spectrophotometer (BIO-2000, Bioplus 2004R, Barueri, SP, Brazil) at 505 nm wavelength to obtain a specific optical density, and the values were converted to glucose concentration. All readings were taken in duplicate. The number of leaked samples for each group was considered for statistical analysis.

Bacterial leakage test

The external surfaces of thirty root segments (15 per group) were made impermeable with two layers of cyanoacrylate adhesive (Araldite) except for the 1 mm around the apical foramen. Following, a 1mL pipette connected to a piece of rubber with a central perforation was adapted on the cervical portion of the canal of each root segment, and the interface was sealed with cyanoacrylate adhesive (Araldite). The assembly was sterilized by ethylene oxide gas (ACECIL, Central de Esterilização Com. Ind, Ltda., Campinas, SP, Brazil) and then adapted

to a sterile 20mL syringe containing 5mL of Brain Heart Infusion broth (BHI), so 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 the sterilization.

For the leakage assays, a standard strain of *E. 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 (Difco Laboratories, Becton Dickinson and Company, Franklin Lakes, NJ, USA) and incubated at 37°C for 24 h. After the incubation period, the number of colony forming units (CFU mL⁻¹) was determined. For assessment of bacterial leakage, 500 μ L aliquots of standard *E. faecalis* were transferred to the upper portion of the pipette connected to the root segment. After every 7 days during the experimental period, the BHI inoculated with *E. faecalis* was replaced with a new 500 μ L aliquot of sterile BHI. The aliquot removed was tested to confirm bacterial viability. Samples were observed daily for 70 days, and leakage was detected by turbidity of the BHI medium in contact with the apical portion of the root segment. The number of leaked samples for each group was considered for statistical analysis.

For the positive control group (n = 2), root segments without apical

plug were used. Two teeth with intact crowns had their root surfaces covered with two layers of nail varnish and were used as negative control group (n = 2). Besides that, one specimen of each experimental group was used as negative control and inoculated with BHI without *E. faecalis*.

The numbers of leaked samples for similar groups in both tests, at study completion, was compared by the Fisher test. The level of significance was set at p < 0.05. The analysis was performed in the software SAS 9.3.

RESULTS

Glucose leakage

At the end of the observation period, no trace of glucose solution was detected in the negative control group, while in the positive control group the entire amount of glucose passed through the canal. The number of specimens with glucose leakage in group 1 was 9 (60%), compared to 5 in group 2 (33.33%) (**Table 2**).

Bacterial leakage

In the negative control group, none of the specimens leaked at the end of 70 days, while all specimens in the positive control group had turbidity of the medium within 24 h. The highest number of specimens showing leakage was observed in group 1 (11 / 78.57%), followed by group 2 (8 / 57.14%). In all cases, the inoculums were confirmed to contain *E. faecalis* (**Table 2**).

Statistical agreement between leakage methods

The percentage of specimens with detectable glucose and bacterial penetration along the apical plugs for each group during the experimental period is shown in **Figure 1**. There was no significant difference between

Table 2. Number and percentage of specimens with and without glucose and bacterial leakage along the apical plugs.

	Method	Leakage		No leakage		Total n	P-value
		n	%	n	%		
1	Glucose	9	60	6	40	15	0.297
	Bacterial	11	78.57	3	21.43	14	
2	Glucose	5	33.33	10	66.67	15	0.211
	Bacterial	8	57.14	6	42.86	14	

tests, both for group 1 ($p = 297$) and group 2 ($p = 0.211$) (Table 2).

DISCUSSION

A high variety of leakage methods have been performed over time in order to analyze the sealing ability of MTA, such as fluid filtration¹³, dye¹⁴, protein¹⁵, endotoxin¹⁶ and glucose leakage^{2,3}, as well as bacterial penetration¹⁷. Taking in account the lack of a standard test and the criticism about the shortcomings related to leakage methods applied in endodontics^{8,9}, a deep analysis of the literature was performed in order to choose the most sensitive, reliable and clinically relevant leakage tests to be compared. Thereby, glucose and bacterial penetration appeared to meet the requirements^{4,18,19} aside from to be some of the most currently used in vitro leakage models^{1,2,3} that determine the presence of voids along the root filling materials or at the interface between material and dentin walls²⁰.

The glucose leakage test is considered a reliable method due to its specificity and sensitivity^{4,18}. In addition, it is useful for quantitative leakage evaluation², and overcoming most limitations observed in other leakage tests⁴. The glucose molecules, nutrient source for bacteria with low molecular weight (180 Da)⁴, are considered a clinically relevant marker for leakage tests². Besides, it has already been proven that, until up to two hours of contact between MTA and glucose, there is no significant glucose reduction².

On the other hand, some authors believe that, among all in vitro methods, the bacterial penetration model is the most clinically relevant and might be more biologically significant¹⁹. It has the advantage of using a biological marker¹⁹, the etiologic agent of apical periodontitis⁵, which may precisely indicate the sealing ability⁶.

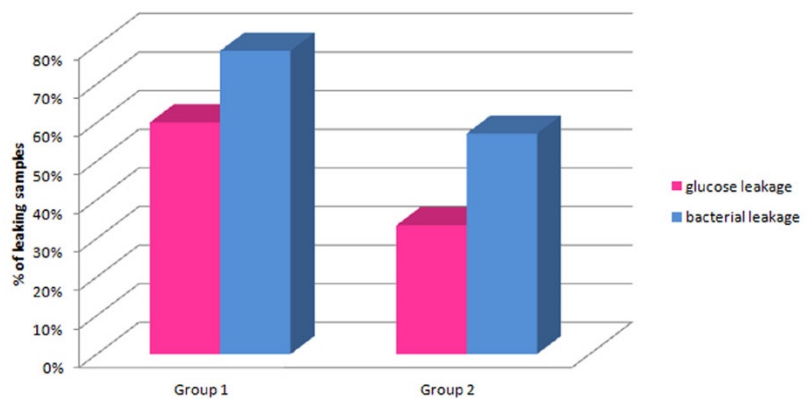


Figure 1. Percentage of specimens with detectable glucose and bacterial penetration along the apical plugs.

Usually, a specific leakage test has been performed aiming to evaluate the sealing ability of several materials or techniques^{1,2,6,13,15,16}. Nevertheless, the results obtained from different leakage models on similar specimens have rarely been compared on the same study^{21,22,23,24}. Therefore, this paper attempted to provide a comparison between results from two different leakage methods, namely glucose and bacterial penetration, which had not been compared so far.

At the end of the experimental period, both tracers penetrated all root segments of the positive control group and none of the negative control group. This indicates that the experimental groups provided a reliable result of leakage, since bacterial and glucose penetration would occur only through voids within the material or between material and dentin walls²⁰.

When experimental groups were analyzed, it was possible to observe a similar pattern of leakage between tests within the same group. Some studies have compared glucose or bacterial penetration to other leakage methodologies^{5,10,22,23,25,26} and conflicting results have been showed. When glucose leakage was compared with fluid transport, it was possible to notice that the glucose test provided the highest sensitivity²², or that both tests were similar²³. No difference was found in the comparison between bacterial penetration and fluid

transport¹⁰. It is possible that there may be little differences in results between tests, such as bacterial and glucose penetration and fluid transport since they work in a similar manner for evaluation of leakage. In contrast, other investigations showed poor correlation between bacterial and dye leakage^{5,25,26}, and among dye leakage, bacterial penetration, electro-chemical test, and fluid filtration²⁷. The lack of agreement and the discrepancy between results may be due to the differences in working principles of various methods and the different materials²⁷. Thus, it must be considered reservation and limitation about the comparability of the results.

Although there was no difference between results from tests within groups 1 and 2, the present experimental study was able to demonstrate that the bacterial leakage test was more sensitive, since the number of specimens showing leakage was superior to the glucose leakage model. Other studies could be considered in agreement with this observation, since bacterial penetration was observed in a higher number of samples compared to dye leakage^{5,25,28}. However, it should be kept in mind that there is difference between particle size of leakage markers²⁹, such as glucose and dye. Considering only the results of the bacterial leakage test, when MTA was used as root-end filling material,

55%²⁵, 91.7%²⁸ and 75%²⁸ of the samples showed *E. faecalis* penetration after 70 days, values close to 78.57% of the present study.

Due to the lack of similar studies in the literature comparing glucose and bacterial leakage methods, discussion of the results remains limited and cannot be properly conducted. It is, therefore, undeniable the claim for standardization of leakage tests^{8,9}.

It is worth noting that the best result of sealing ability in both leakage models was obtained when PBS was used as intracanal dressing. Recent investigations have demonstrated that the interaction of MTA with PBS positively influences its sealing ability^{3,13,30}. This occurs due to the release of some components of MTA when in contact with PBS, triggering the initial precipitation of amorphous calcium phosphates, which act as precursors for the formation of carbonated apatite. The formed apatite leads to formation of an interfacial layer with intratubular mineralization at the biomaterial-dentin interface¹², which improve the sealing.

It is known that the results obtained from ex vivo studies might not be directly extrapolated to the clinical situation²⁴. To solve the problem, many efforts have been done to standardize and validate the leakage tests^{8,9}. Nevertheless, while the relationship between results of an ex vivo leakage model with clinical success or failure of endodontic treatment remains undetermined, it is very useful to know which type of leakage method provides more reliable results when the effectiveness of endodontic materials or techniques must be evaluated.

CONCLUSION

Within the parameters of the present study and regardless limitations, the results of the present ex vivo study allowed concluding that there was no difference between

glucose and bacteria leakage evaluation methods.

CONFLICT OF INTEREST

The authors deny any potential conflict of interests.

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