

Potential sealing ability of a new propolis-based sealer used as apical plug compared to MTA

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ABSTRACT

Objective: The main objective of this study was to evaluate and compare the sealing ability of an experimental propolis-based cement (EPC) and mineral trioxide aggregate (MTA), both as apical plug, using the glucose leakage system. Thereafter, a bacterial leakage test was employed to evaluate the EPC sealing ability. **Methods:** For the glucose leakage test, 30 root segments (12 mm) were randomly divided into two experimental groups ($n = 15$), according to the cement used as apical plug, as follows: G1 = MTA; G2 = EPC. Root segments were prepared and the amount of glucose leakage was measured, following an enzymatic reaction, and quantified by a spectrophotometer. Data were analyzed by means of Student's t-test

($p < 0.05$). For the bacterial leakage test, another 15 teeth were subject to apexification procedures, filled with EPC and mounted in a specific apparatus. The number of leaking specimens was observed weekly for 70 days. **Results:** EPC was significantly superior to MTA ($p < 0.001$). The EPC sealing ability was 100% and 60% efficient in glucose and bacterial leakage tests, respectively. **Conclusions:** The experimental propolis-based cement used as apical plug promoted superior sealing ability when compared to MTA, and excellent performance in both leakage systems.

Keywords: Apexification. Dental leakage. Glucose. *Enterococcus faecalis*. Propolis.

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Introduction

Mineral trioxide aggregate (MTA) used as an artificial apical barrier allows compression of the filling material^{1,2} and restricts or prevents the penetration of microorganisms and toxins from the root canal into the periapical tissues.³ In addition, it creates a favorable environment for the deposition of calcified tissue over its surface and in continuity with the previous apical cementum.⁴

The sealing ability of MTA is superior to that of other types of material.^{5,6} Notwithstanding, few studies demonstrate totally effective sealing when MTA is used as a retrograde filling material or as an apical plug.^{7,8} Taking into account that the penetration of microorganisms through the filling material or at the dentin/material interface compromises treatment success,⁹ and that MTA is unable to ideally promote sealing, more research is needed to discover or develop material with superior sealing ability than the dental materials already known.

Propolis, also referred to as “bee glue,” is a multifunctional material that has been used in Medicine for years due to its anti-inflammatory, antimicrobial, antioxidant¹⁰ and antifungal properties.¹¹ In general, propolis is composed of resin and vegetable balsam (50%), wax (30%), aromatic and essential oils (10%), pollen (5%), and other substances (5%).^{12,13} Since it is a resinous substance, propolis is collected from various plants and used by honeybees in the construction and maintenance of their hives, as well as for sealing cracks or gaps in honeycombs, providing protection against intruders.¹¹ Currently, propolis has been tested in Dentistry as a transport medium for avulsed teeth,¹⁴ endodontic disinfectant,^{15,16} and intracanal dressing.^{14,17} One study demonstrated that propolis is biocompatible and favorable to periapical tissue repair.¹⁷

Previous pilot experiments showed that using a specific sort of *in natura* propolis as apical plug is not suitable, since it has solid consistency and it is difficult to manipulate. Nonetheless, when heated to 37°C, it acquires higher viscosity and better work conditions, allowing its utilization. Propolis composition and consistency, and the possibility to provide effective sealing, encouraged the development of an experimental propolis-based cement (EPC) to be used in different clinical situations, including apexification procedures. Thus, the main objective of this study was to evaluate and compare the sealing ability of EPC and MTA, as apical plugs, using the glucose leakage method.

Material and Methods

The study was approved by the Ethics Committee for Research with Human Beings of Universidade Federal de Santa Catarina (protocol #228.898).

Experiment 1

Thirty-four extracted human single-rooted teeth were used. The procedures were performed as previously described by Almeida et al.¹⁸ The crowns of 32 teeth 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 with #1 to #5 Gates-Glidden drills in a crown-down fashion, and 1% sodium hypochlorite (NaOCl) was used for irrigation. A standardized open apex was created by retrograde preparation of the canal with a #6 Gates-Glidden drill (± 1.50 -mm diameter). Final canal rinse was performed with 17% EDTA followed by 1% NaOCl.

Apexification procedures

The root sections were randomly divided into two experimental groups ($n = 15$), according to the material used as apical plug: G1) MTA – mixed following the manufacturer's recommendations; G2) EPC – heated to 37°C and manipulated.

Both cements were 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 cements placement and plug thickness. In the MTA group, a cotton pellet moistened with distilled water was placed in the cervical region of each root segment. It was replaced by a dry pellet after 24 hours. In the EPC group, the cervical region remained empty. All access openings were filled with Cimpat (Septodont Brasil, São Paulo, SP, Brazil). Thereafter, the root segments were introduced into plastic vials containing floral foam moistened with 20 ml distilled water and stored for seven days at 37°C to ensure setting. After this period, the external surface of all specimens was made impermeable with two layers of nail varnish, except for the 1 mm around the apical foramen.

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 ($n = 2$).

Assembled double chamber and glucose leakage measuring

The root segments were secured to a device designed to test glucose leakage (similar to that described by Almeida et al¹⁸). The cervical portion of each root segment was fastened to 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 water. Low-viscosity cyanoacrylate adhesive (Araldite, Brascola, Joinville, SC, Brazil) was used to seal all interfaces and connections.

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

Table 1. Groups, number and percentage of samples with traces of glucose solution and concentration mean value.

| Groups | n (%) | Glucose (g x L ⁻¹) |
|--------|---------|--------------------------------|
| 1 MTA | 12 (80) | 4.69 |
| 2 EPC | 0 (0) | 0 |

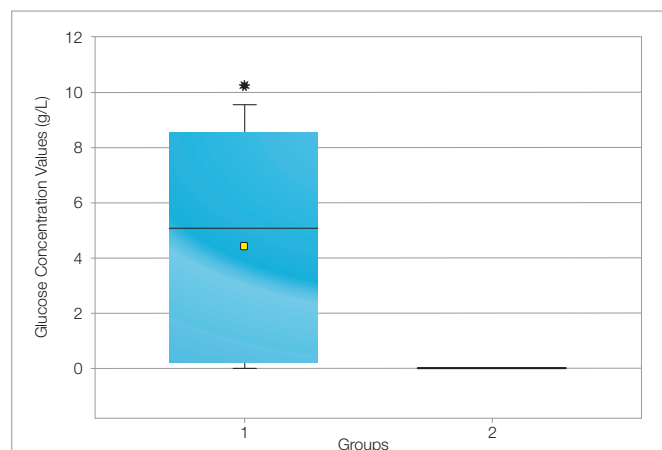


Figure 1. Box plots of glucose concentration values in each group, illustrating the mean traces, minimal and maximal glucose traces and the median.

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

Each sample 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, and the mean value was considered for statistical analysis. Data were analyzed statistically by Student's t-test at a significance level of 5%.

RESULTS

Control groups

In the negative control group, no trace of glucose solution was detected; whereas in the positive control group, mean glucose concentration was 88.16 g x L⁻¹.

Experimental groups

The number and percentage of samples that showed traces of solution, as well as the mean values of glucose concentration are shown in Table 1. The sealing ability of EPC was significantly higher than that presented by MTA ($p < 0.001$). The graph in Figure 1 displays the mean, median, standard deviations and data distribution for each experimental group.

Since EPC demonstrated notable sealing ability, a complementary bacterial leakage test was conducted to confirm the obtained results.

Experiment 2

Another 15 teeth were submitted to the apexification procedures and filled with EPC, as described above. The root segments were introduced in plastic vials containing floral foam moistened with 20 ml distilled water and stored for seven days at 37 °C to ensure setting. After this period, the external surface of all specimens was made impermeable with two layers of nail varnish, except for the 1 mm around the apical foramen. An apparatus with the root segment was mounted (similar to the model developed by De Leimburg, et al⁴), sterilized by ethylene oxide gas (ACECIL, Central de Esterilização Com. Ind. Ltda., Campinas, SP, Brazil), and adapted to a sterile

20-ml syringe containing 5 ml of Brain Heart Infusion broth (BHI). Thus, the most apical portion of each root segment was immersed into the culture medium. The syringe embolus allowed closure of the apparatus which was kept in an oven at 37 °C for four days to ensure sterilization.

For the positive control group, two root segments without apical plug were used. For the negative control group, two root segments with EPC apical plugs were made completely impermeable by the application of two layers of nail varnish.

Bacterial leakage test

For the bacterial leakage assay, a standard strain of *Enterococcus faecalis* (ATCC 29212) was used. For the execution of the bacterial leakage test, 500 µl aliquots of an overnight culture ($\approx 10^8$ colony forming units/ml) were transferred to the upper portion of the pipette tip connected to the root segment. After every seven days during the experimental period, the BHI inoculated with *E. faecalis* was replaced with a new 500-µl aliquot of sterile BHI. The aliquot was removed and tested to confirm bacterial viability. The number of leaking specimens was observed weekly for 70 days. Leakage was detected by turbidity of the BHI medium in contact with the apical portion of the root segment.

RESULTS

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

EPC group

The number of specimens with bacterial leakage at the end of each week is listed in Table 2. A total of nine EPC apical plugs promoted effective sealing up to 70 days.

Table 2. Frequency of bacterial leakage over the 70-day experimental period.

| Specimens with bacterial leakage (n) | | | | | | | | | | |
|--------------------------------------|-------|---|---|---|---|---|---|---|---|----|
| Group | Weeks | | | | | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| EPC | 0 | 0 | 0 | 0 | 1 | 1 | 2 | 0 | 1 | 1 |

Discussion

The glucose leakage test under pressure (experiment 1) is useful for quantitative evaluation,¹⁹ has a high degree of specificity and sensitivity,^{20,21} and overcomes most limitations observed in other leakage tests.²¹ Furthermore, glucose is considered more relevant than others markers, due to its low molecular weight (180 g/mol).²¹ The pressure of 103 KPa, applied in previous experiments,^{18,19} does not damage the structure of the cement¹⁹ and reduces testing time in comparison to the original glucose leakage model.²¹ Thus, up to one hour of contact between MTA or EPC with glucose, there is no glucose reduction, as verified in a pilot experiment (developed according to the methodology proposed by Leal et al¹⁹).

The bacterial leakage test (experiment 2), in turn, reflects clinical reality, since it has the advantage of using the etiologic agent of apical periodontitis²² and may provide a precise indicator of sealing ability.

In the first experiment, the majority of samples in Group 1 showed traces of glucose, indicating failure in the sealing ability of MTA. Other studies showed similar results when the percentage of leaking specimens was evaluated.²⁴ Probably, the occurrence of glucose leakage was due to the presence of through-and-through voids in the cement mass or at the cement-dentin interface.¹⁹ The presence of interconnected pores in the body of the material²⁵ may also have allowed leakage.

EPC apical plugs prevented leakage in a remarkable value of 100% in experiment 1 and 60% in experiment 2. A possible reason to justify the sealing ability promoted by the propolis-based cement in both leakage tests may be its essentially resinous composition, which provides it with a strong adhesive characteristic.¹¹

Even though some specimens allowed bacterial leakage up to 70 days, the results obtained can be considered satisfactory. It is possible that the prolonged contact of the apical plug with the culture medium weakened and negatively altered propolis adhesion to dentin walls over time, allowing the occurrence of leakage along the EPC apical plug, since turbidity of the BHI medium was detected only after the fifth week.

As EPC is an experimental cement, and due to lack of similar materials reported in the literature, discussion of results remains limited and cannot be deeply conducted. Notwithstanding, due to the excellent results obtained, there is a perspective of using

this cement not only as apical plug, but in all cases in which effective sealing is needed. However, it is important to emphasize that the present experiment is part of a preliminary study with EPC. There are other ongoing tests to evaluate its physicochemical and biological properties.

Conclusions

Under the conditions of the present study, it was possible to conclude that the experimental propolis-based cement used as apical plug promoted remarkably superior sealing ability compared to MTA, and excellent performance in both leakage systems.

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