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Ex Vivo Evaluation of Bacterial Leakage and Coronal Sealing Capacity of Six Materials in Endodontically Treated Teeth

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Introduction: Successful endodontic treatment requires an effective coronal sealing to prevent the penetration of saliva and microorganisms into the root canal system. We aimed to investigate the sealing capacity of Maxxion R, Intermediate Restorative Material (IRM), Mineral Trioxide Aggregate-like material (Biodentine), White Cimpat, Flow Resin and Z250 Resin against *Enterococcus* (*E.*) *faecalis* infiltrates, when used as coronalsealants after endodontic treatment. **Materials and Methods:** Sixty-six roots of adult lower premolars were randomly divided into 6 experimental groups with 10 roots each (n=10), and two control groups (positive and negative) with three roots each. The root canals were instrumented to ProTaper F3 file, irrigated with 2.5% NaOCl and 17% EDTA, and filled using Tagger's Hybrid technique with AH-Plus cement. After removing 2 mm of the coronal third filling with a Gates Glidden #6 drill, the cervical portion of each of the sixty roots was sealed with a 2 mm-thick plug, plus the respective material being tested in this study. All roots were fitted to silicone devices (Eppendorf) with cut extremities and sterilized with ethylene oxide; experimental procedures were performed in a laminar flow chamber for aseptic chain maintenance. All specimens were inoculated with *E. faecalis*, and the culture medium was renewed every 3 days for 60 days. Medium turbidity was evaluated daily. The obtained data were subsequently submitted to analysis of variance (ANOVA-R) complemented by Student's *t*-test at a significance level of 5%. Analyzes of variance were calculated using the SAS system GLIMMIX procedure. **Results:** Biodentine (56.90), Z250 Resin (54.90) and White Cimpat (53.30) resisted contamination for a longer time compared to Maxxion R (51.30), Flow Resin (50.70), and IRM (48.70) over a period of 60 days. **Conclusion**: Biodentine, Resin Z 250 and White Cimpat presented the lowest infiltration averages when compared to the other tested materials.

Keywords: Coronal Sealing; Dental Pulp Cavity; *Enterococcus faecalis*; Ethylene Oxide; MTA-like Materials

Introduction

Endodontic therapy aims to restore physiological normality to dental elements. Its success, however, depends on several steps; from the access surgery phase to the restoration of the dental element itself. Considering the large percentage of coronal microleakage-induced endodontic treatment failures, an adequate three-dimensional sealing is indicated, with the coronal portion being particularly important [1].

Several factors may lead to coronal microleakage, including premature loss of the temporary restoration, or an inadequate final restoration. Microleakage allows the oral microbiota to enter into the root canal system, which can cause the endodontic treatment to fail. Exposure of gutta-percha to saliva in the coronal chamber leads to the migration of bacteria and toxins towards the apex within a few days or hours, respectively. According to Schwartz and Fransman [2], the root canal entrance orifice constitutes a second line of defense against microbial infiltration. Yamauchi *et al.*[3], in an *in vivo* study, reported a considerable reduction of apical periodontitis when a coronal plug was used.

These observations have granted particular importance in

recent years to the immediate coronal sealing of the pulp chamber, post endodontic treatment. With the goal of avoiding crown-apex leakage, Jenkins *et al.* [4], compared several temporary and definitive restorative materials resinous, ionomeric, and adhesive systems, placed on recently treated teeth, and found the addition of a barrier between the oral medium and the root canal system (at a depth still to be determined) appears to decrease leakage. Therefore, a cervical plug in the canal orifice could prevent microleakage and extend the leakage-free period before restorative treatment is initiated [5, 6].

The existing literature has yet to describe a single material comprising all the physical properties necessary for a good coronal seal that avoids bacterial infiltration. Therefore, new materials need to be evaluated so that their performance can be measured.

Many studies have shown conflicting results on the sealing ability of different temporary restorative materials, which could be due to the different methods used in these studies, especially with regard to the techniques used to measure coronal microleakage over different time periods [7-9]. The aim of this *in vitro* study was to investigate the sealing capacity of temporary filing materials when used as coronal sealants after endodontic treatment.

Materials and Methods

The present study was approved by the institutional research ethics committee (2010/0159). Teeth were donated by the Faculdade São Leopoldo Mandic.

Sixty-six human adult monoradicular inferior premolars from the Human Teeth Bank of the São Leopoldo Mandic Graduate Center-Campinas-SP were used. Roots were straight, fully formed, with lengths between 14 mm and 16 mm, without previous endodontic treatment, absence of calcification, and an anatomical diameter between K-files sizes 15 and 20 (Mani, Tochigi, Japan). After tooth extraction, specimens were cleaned with periodontal curettes and stored in 0.9% saline solution. Teeth crowns were sectioned with a 1757 diamond bur (KG Sorensen, Barueri, SP, Brazil) operating at a high rotational speed, to standardize at 14-16 mm long roots. Canals were instrumented with ProTaper intruments (Dentsply Maillefer, Ballaigues, Switzerland) up to F3 file (30/0.09), irrigated with 5 mL of 2.5% NaOCl between filings. After instrumentation, specimens were irrigated with a total of 3 mL of 17% EDTA, rinsed with saline and subjected to three 20 sec-long ultrasound cycles (totaling one min), followed by another irrigation with a total of 6 mL of 2.5% NaOCl and a further three 20 sec ultrasound cycles. Subsequently, the teeth were irrigated with 5 mL of 0.9% sodium shloride, dried with capillary suction tips and #30 absorbent paper cones, and filled with gutta-percha and AH-plus endodontic cement (Dentsply, Tulsa Dental, Tulsa, OK, USA) using Tagger's hybrid technique [10]. Shortly thereafter, the cavity was prepared by removing 2 mm of gutta-percha from the cervical portion with a Gates-Glidden #6 drill (Mani, Tochigi, Japan). The sixty roots were randomly distributed using the Random program (www.random.org) into 6 groups with 10 specimens each (for experimental groups 1 to 6, n=10).

The teeth in each group had their cervical portion sealed with a 2 mm-thick plug with the following materials: *1.* Group IRM, intermediate restorative material (IRM) (Dentsply, Tulsa Dental, Tulsa, OK, USA); *2.* Group Maxxion R, glass ionomer cement (Maxxion R; FGM , Joinville, Brasil); *3.* Group MTA-like materials, Biodentine (Septodont, Saint-Maur-des-Fosses, France), *4.* Group R, Z250 resin (3M ESPE Adper, St. Paul, MN, USA); *5.* Group C, temporary cement (White Cimpat; Septodont, France) and *6.* Group FR, flow resin (3M ESPE Adper, St. Paul, MN, USA). All materials were placed according to the manufacturer's specifications.

There were also two control groups: *A.* Positive control group (n=3); roots having instrumented canals only; and *B.* Negative control group (n=3): intact teeth.

The roots of the experimental groups and controls were stored for 48 h at 37°C in a humidified oven to allow the canal sealing materials to set. Roots were subsequently waterproofed with 2 layers of cosmetic enamel (Impala, Guarulhos SP, Brazil), except for a 2 mm portion just before the apex. To perform the leakage test, a device was assembled for each sample, consisting of Eppendorf tubes (Cral, Comércio de Artigos para Laboratório Ltda., São Paulo, Brazil) with cut extremities, to which the roots were internally adapted so that 2 mm of their length remained outside of the tube. The junction between the root and the tube was sealed with a fast polymerization epoxy resin (Araldite, Brascola Ltda, São Paulo, SP, Brazil) (Figure 1). All Eppendorf and specimen sets were briefly sterilized with ethylene oxide.

All procedures described henceforth were performed inside the laminar flow chamber of a microbiology laboratory to maintain the aseptic chain. For microbiological analysis, the Eppendorf tubes and specimens were inserted into 13 mL glass bottles, containing 9 mL of Heart Infusion culture broth (BHI) (Merck, Darmstadt, Germany). To prepare the inoculum, 100 µL of the *Enterococcus* (*E.*) *faecalis* stock was combined with 2 mL of brain-heart infusion broth (BHI, Merck, Darmstadt, Germany) and kept in an oven at 37°C for up to 24 h, until the broth exhibited turbidity.The specimens from the experimental groups and control groups received E. *faecalis* inoculum adjusted

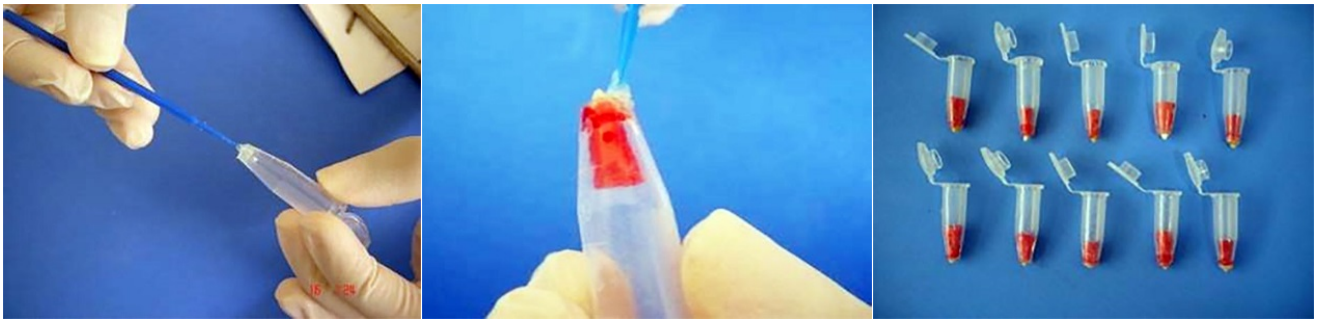

Figure 1. View of specimens inside the Eppendorf tube

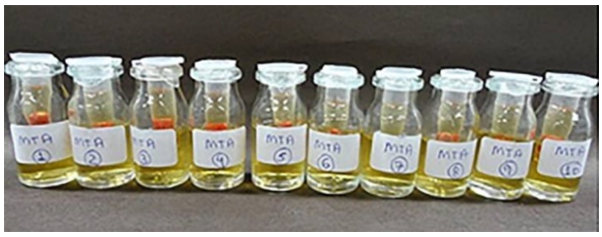

Figure 2. MTA like material (Biodentine) Group

to McFarland scale 2; culture medium was renewed every 3 days for a period of 60 days.Flasks were evaluated daily for a pre-determined 60-day period to verify the turbidity of the medium in the lower chamber of the apparatus. All observations were recorded until the contents were submitted for microbiological tests (Figure 2). When turbidity was observed in the medium, the apparatus was disassembled to collect a sample to be seeded in m-Enterococcus solid medium (Nutrient broth, NB, Difco Laboratories, Detroit, MI, USA) and incubated for 24 h. To analyze the growth of *E. faecalis* and verify the absence of external contamination, Gram staining and catalase tests were performed.

Statistical analysis

One-way analysis of variance on ranks was applied, calculated using the SAS system's GLIMMIX procedure (SAS Institute Inc. The SAS system, release 9.2. SAS Institute Inc., Cary NC, USA, 2009). The student's *t*-test was chosen to perform multiple comparisons of means, at a 5% significance level for all tests.

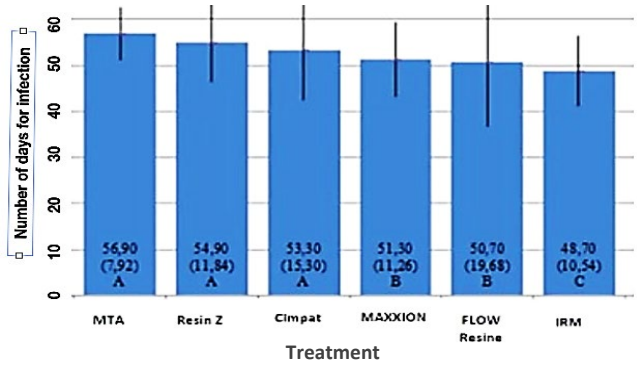

Figure 3. Mean (SD), confidence interval limits of the mean (95%), and Student's t-test (=0.05). Bars with the same letters indicate averages that do not differ significantly from one another

Results

Student's *t*-test indicates the formation of 4 treatment groups with significantly different means. One group, formed by the treatments Negative control (60.00), Biodentine (56.90), Z250 Resin (54.90), and White Cimpat (53.30), exhibited a longer time until leakage. A second group, with lower mean values than the first, comprised the Maxxion R (51.30) and Flow Resin (50.70) treatments. Finally, IRM (48.70) exhibited a significantly higher mean value only when compared to positive control (1.00). The comparisons are shown in Figure 3 and Table 1.

Table 1. Mean (number of observations), standard deviation, limits of confidence intervals for the mean (95%) and Student's t test for comparing means with a significance level of 5%

Group	Mean (SD)	Mean confidence limit		Student's t test (P=0.05)
		Superior	Inferior	
Biodentine	56.90 (7.92)	62.57	51.23	A
Z 250	54.90 (11.84)	63.37	46.43	A
Cimpat	53.30 (15.30)	64.25	42.35	A
Maxxion R	51.30 (11.26)	59.36	43.24	B
Flow	50.70 (19.68)	64.77	36.63	B
IRM	48.70 (10.54)	56.24	41.16	C

Means with the same letters do not differ significantly from each other

Discussion

The aim of this study was to search for ways minimize the deleterious effects of leakage in endodontic treatments, and consequently improve the health of dental patients. To accomplish this, we developed an *ex vivo* test to evaluate the occurrence of coronal leakage through various restorative materials. Additionally, we tested clinical solutions suggested in other *ex vivo* [11-13] and *in vivo* [14, 15] studies.

The sterilization process preserve the properties of the enamel used in *in vitro* and *in situ* research by Viana [15]. Ethylene oxide vapor was thus chosen (in agreement with the aforementioned citation) for its adequate sterilization of the specimens [16] as it reacts with microorganism constituents containing nucleic acids and functional proteins. Moreover, the vapor does not significantly alter enamel and dentin microhardness nor result in demineralization. In the DES/RE test [17], its effect on the teeth was negligible, but Viana [15] stated that cracks can be observed on the surface of the tooth, which meant that some specimens needed to be excluded.

Culture medium turbidity was evaluated in the periods of observation according to Estrela *et al.* [13]. Turbidity would indicate microbial leakage at the restorative material/coronal cavity interface. The experimental period was extended for 60 days, in line with the work of Fathi *et al.* [12]. This amount of time proved sufficient to observe differences between the materials tested. Estrela *et al.* [13] divided specimens into five groups of ten teeth each, which were observed after 7, 21, 30, 45 and 60 days. Result analysis showed that IRM- or Cavit-restored specimens suffered leakage after just 7 days.

After application of the Kruskal-Wallis test, the IRM treatment exhibited the highest leakage mean values. These results are in agreement with the majority of the existing literature, wherein IRM exhibits high leakage and poor sealing values [18-20].

Flow Resin was not as efficient as glass ionomer cement as a canal-filling base, corroborating the findings of Çelik et al. [11] Likewise, Sauáia *et al.* [20] found that fluid resin had the worst results for microleakage, which can be explained by the high polymerization contraction this type of resin does not contain a filler.

In the present study, White Cimpat showed good provisional seal results, in agreement with previous studies [21]. However, these results are in contrast with those of another study which showed that White Cimpat and Coltosol cements presented the highest marginal leakage values of nickel ions, in a way that was statistically similar between each other and the positive control.

MTA sealant is also being increasingly studied. Tselnik *et al.* [22] evaluated MTA White, MTA Gray, and glass ionomer modified resin as leakage preventing coronal barriers, using a 3mm thickness in the samples. The results were not statistically significant among the samples. They recommended a 3 mm intracoronal barrier. However, the present study corroborates Mah *et al.* [23] who stated that a 2 mm thickness was sufficient in preventing oral microorganism leakage for 10 months or more.

Endodontics has benefited from adhesive procedures for coronal sealing [24]. Thus, 3M's dentinal adhesive and composite resin Z250 were also evaluated. The latter presented better sealing *properties* than both IRM and glass ionomer cement (Maxxion R), in agreement with Couto *et al.* [25], and seem to be more appropriate for coronal plugging in endodontics.

The results obtained herein and in the aforementioned literature are in *agreement* with Cortez *et al.* [26] who stated that gutta-percha + sealant is not completely able to prevent coronal microleakage of endodontically treated teeth. Thus, the need for a good restorative material used as a coronal plug becomes evident, which in conjunction with the permanent restoration can prevent microleakage.

Conclusion

Based on the methodology used and the results obtained, Biodentine, Z250 resin and White Cimpat delayed contamination for a longer time when compared to the other materials tested: Maxxion R, Flow Resin, and IRM, over a period of 60 days.

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Conflict of Interest: 'None declared'.

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