10.5005/jp-journals-10024-1663

ORIGINAL RESEARCH

Sealing Ability of Root-end Filling Materials

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ABSTRACT

Background: The aim of this research was to compare the apical sealing ability of different root-end filling materials (Super EBA®, ProRoot MTA®, thermoplasticized gutta-percha + AH-Plus®, thermoplasticized RealSeal®), by means of microbial indicators.

Materials and methods: Thus, 50 human single-rooted teeth were employed, which were shaped until size 50, retro-prepared with ultrasonic tips and assigned to 4 groups, retro-filled with each material or controls. A platform was employed, which was split in two halves: upper chamber—where the microbial suspension containing the biological indicators was introduced (*E. faecalis* + *S. aureus* + *P. aeruginosa* + *B. subtilis* + *C. albicans*); and a lower chamber containing the culture medium brain, heart infusion, where 3 mm of the apical region of teeth were kept immersed. Lectures were made daily for 60 days, using the turbidity of the culture medium as indicative of microbial contamination. Statistical analyses were carried out at 5% level of significance.

Results: The results showed microbial leakage at least in some specimens in all of the groups. RealSeal® has more microbial leakage, statistically significant, compared to ProRoot® MTA and SuperEBA®. No significant differences were observed when compared ProRoot® MTA and SuperEBA®. The gutta-percha + AH Plus results showed no statistically significant differences when compared with the other groups.

Conclusions: All the tested materials showed microbial leakage. Root-end fillings with Super-EBA or MTA had the lowest bacterial filtration and RealSeal shows highest bacterial filtration.

Keywords: Apical surgery, Microbial leakage, MTA, Resilon, Root-end filling materials, Super EBA.

How to cite this article: Amezcua O, Gonzalez AC, Borges AH, Bandeca MC, de Araújo Estrela CR, Estrela C. Sealing Ability of Root-end Filling Materials. J Contemp Dent Pract 2015;16(3):210-214.

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Source of support: Nil

Conflict of interest: None

INTRODUCTION

Persistent endodontic infections in filled root canals are among the main causes of root canal failure.[1] Because of the microbial etiology in these cases, it is mandatory to decrease the microbial load. In the reintervention cases this is achieved by means of the instrumentation-irrigation-root canal dressing protocols that have shown predictable results.[2] There are reports on root canal reintervention showing failure on 10 to 38% of the cases.[3] The main reason reported on those failled cases is due to the presence of bacteria organized as biofilm in the root canal system.[1] The mechanical debridement in diameter and length of the infected root canal plays an important role to decrease the microbial load.[4] The other resource to prevent remaining bacteria reach into periapical tissues is the root-end filling, therefore, the sealing ability of the chosen material plays an essential role.[5]

In order to know their physico-chemical properties, the sealing ability of different root-end filling materials such as Super-EBA, mineral trioxide aggregate (MTA) and termoplasticized gutta-percha have been evaluated by different metodologies.[5-11] Mineral trioxide aggregate is indicated because of its excellent physical, chemical, and biological properties.[12-14] On the other hand, it presents a long setting time, high cost, poor adhesion to dentin and low resistance to compression.[15]

AH Plus (Dentsply DeTrey, Konstanz, Germany) is a hydrophobic epoxy resin-based sealer that is been used as the gold standard for comparisons with other endodontic sealers.[16] Considering the stability, this material presents smaller dimensional changes. Its sealing ability is compromised in function of the difficulty to bond to gutta percha and in the presence of moisture, the material does no efficiently adhere to canal walls.[17,18] Recently, there have been introduced to endodontics materials aiming to have dentin adhesion, such as Resilon (Real Seal®, Sybron Endo, Glendora, USA) which is a polymeric resin having the cones and the sealer a similar chemical composition. It uses a dentine etch to produce an hybrid layer between the fluid resin and dentine. Its indication as root canal filling makes relevant to study under the same conditions that gutapercha is used.[19]

The aim of this study is to evaluate the microbial leakage in different root-end filling materials (Super EBA®, ProRoot MTA®, thermoplasticized gutapercha + AH Plus® sealer, thermoplasticized RealSeal®).

MATERIALS AND METHODS

Tooth Preparation

Fifty maxillary anterior human teeth without fissure lines in their roots, extracted no more than 90 days before for different reasons and maintained in a humid environment, were selected for this study. Preoperative mesiodistal and buccolingual radiographs of each root were taken to verify the existence of a single canal, absence of internal or external resorption or calcification, instrumented or filled canals and a fully formed apex. The teeth were removed from storage in 0.2% thymol solution and were immersed in 5% sodium hypochlorite (NaOCl; Fitofarma, Lt 30558, Goiânia, GO, Brazil) for 30 minutes to remove organic tissue. The crowns were removed and teeth length was standardized to 16 mm (from root apex to coronal reference). After initial radiographs, standard access cavities were prepared and the cervical third of the canals was enlarged with ISO size 70 to ISO size 90 Gates-Glidden drills (Dentsply/Maillefer, Ballaigues, Switzerland). The canals were prepared up to an ISO size 50 K-File (Dentsply/Maillefer) 1 mm short of the apical foramen. During instrumentation, root canals were irrigated with 2 ml of 1% NaOCl (Fitofarma) at each change of file. Root canals were dried and filled with 17% EDTA (pH 7.2) (Biodinâmica, Ibiporã, PR, Brazil) for 3 minutes for smear layer removal. Thereafter, under a continuous air/water spray, the apical 3 mm of each root was cut-off perpendicular to the long axis of the tooth with a fissure diamond bur (4138G KG Sorensen, São Paulo, Brasil), in a high-speed handpiece. A 3 mm deep root-end cavity was prepared with ultrasonic tip number 1, powered by an ultrasonic unit (Dabi-Atlante, Profi Plus, Riberão Preto, Brasil) and continous irrigation with saline solution. After cleaning and shaping, the root canals were autoclaved for 30 minutes at 121ºC, with all the components. Thus, a perfect microbial control can be obtained without changing the dental structure or damaging the leakage platform.

Experimental Groups

The teeth were randomly assigned to 4 groups of 10 roots each and two control groups (negative and positive, five each), according to the materials tested (Table 1). To standardize root-end fillings, sterilized gutta-percha points size 80 were adapted 3 mm short of the apex in all specimens. The materials were prepared according to the manufacturer's directions and the root-end cavities were filled. Five specimens were filled with Super EBA cement and totally impermeabilized (negative control) and 5 specimens were not root-end filled (positive control). To allow complete set of tested materials, teeth were placed 48 hours at 37ºC in 100% humidity environment.

Test Organisms

This experiment used a mixture of five microorganisms, four reference bacterial strains and one yeast strain, obtained from the American Type Culture Collection. Facultative bacteria included were *Staphylococcus aureus* (ATCC 6538), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 27853), and *Bacillus subtilis* (ATCC 6633). The yeast used was *Candida albicans* (ATCC 10231).

The microorganisms were inoculated in 7 mL of Brain Heart Infusion (BHI; Difco Laboratories, Detroit, MI, USA) broth and incubated at 37°C for 24 hours. The

Table 1: Periods (in days) and medium point where microbial leakage (turbidity) was founded through the materials

	Periods (days)			
Materials	*n*	*Minimum*	*Maximum*	*Medial point*
SuperEBA®	10	28	> 60	24.45^{A}
RealSeal® thermoplasticized	09	02	28	06.89^{B}
ProRoot® MTA	10	41	> 60	23.45^{A}
Thermoplasticized gutta-percha + AH Plus®	07	06	> 60	$17.86^{A.B}$

Different superscript letters represent statistically significant difference ($p < 0.05$)

Table 2: Statistical analysis to evaluate microbial leakage among root-end filling materials

Materials	*p*
SuperEBA® *vs* RealSeal® thermoplasticized	0.000*
SuperEBA® *vs* ProRoot® MTA	0.655
SuperEBA® *vs* thermoplasticized gutta-percha + AH Plus®	0.203
RealSeal® thermoplasticized *vs* ProRoot® MTA	0.000
RealSeal® thermoplasticized *vs* thermoplasticized gutta-percha + AH Plus®	0.107
ProRoot® MTA *vs* thermoplasticized gutta-percha + AH Plus®	0.304

*Mann-Whitney test, Statistical difference when $p < 0.05$

experimental suspensions were prepared by cultivation of the biological indicators on the surface of Brain Heart Infusion Agar (BHIA; Difco Laboratories, Detroit, MI, USA), following the same incubation conditions; microbial cells were resuspended in saline to give a final concentration of about 3×10^8 cells/ml, adjusted to No. 1 MacFarland turbidity scale. One mL of each of these pure suspensions was used to obtain a mixture of the tested microorganisms.

Microbial Leakage Test

In the experimental model, a split platform (upper and lower chamber) was used. In the upper chamber, there was a microbial suspension with the biological markers while the lower chamber contained a culture medium. The microbial mixture could only reach the lower chamber by leaking through the root-end filling.

The coronal portion of the root canal of each tooth was connected to the cut end of a 1.5 mL polypropylene Eppendorf tube (Cral, São Paulo, SP, Brazil) using a cyanoacrylate adhesive (Super Bonder, Itapevi, SP, Brazil) and epoxy resin (Durepoxi, São Paulo, SP, Brazil) to prevent leakage in the junction. The tooth-tube junction was entirely coated with two layers of nail polish (Max Factor, Cosmetics and Fragrances, Los Angeles, CA, USA), except for the apical 3 mm of the root. The teeth used as negative controls were completely coated with two layers of nail varnish including the apical portion of the tube. The specimens (teeth coupled to the polypropylene tubes) were sterilized in 5% NaOCl for 30 minutes and then rinsed with sterile water for 30 minutes.

The polypropylene tubes were attached to a rubber cover that was placed into a 10-mL sterile glass flask containing the culture medium. The flasks were filled with 8 mL BHI broth (Difco, Detroit, MI, USA) with two neutralizers, sodium thiosulfate and Tween 80 both 1%, in such way that 3 mm of the root apex were immersed in the broth. The specimens were placed into the culture medium (BHI) and, to ensure sterilization, the testing apparatus was incubated at 37°C for 24 hours. Teeth that after one incubation day showed bacteriological contamination of culture medium were excluded.

Bacterial Inoculation

The whole apparatus was incubated at 37°C. Fresh overnight cultures of microorganisms were added to the tubes at 7-day intervals. Figure 1 shows a schematic presentation of the MLT apparatus. Microbial leakage was assessed daily for 60 days by two blind calibrated evaluators, having as reference the turbidity of the culture medium, which was considered an indicator of microbial contamination. Positive BHI tubes were selected and inocula were spread on BHI agar surface under identical incubation conditions. To confirm that bacteria present in the positive specimens were the same inoculated previously, Gram stains of the BHI growth and from colonies growing on BHI agar were carried out.

Statistical analysis was carried out for reveal significant differences among the groups (materials) using ANOVA test at 5% level of significance. When sample distribution was non-normal, nonparametric analysis of variance were performed with Kruskal-Wallis test (α = 0.05) and the Mann-Whitney. The tests were performed with the SPSS for Windows statistical software version 12.0.1 (SPSS Inc, Chicago, USA).

RESULTS

Table 1 presents the minimum and maximum periods (days) in which microbial leakage occurred.

Table 2 indicates the difference among the materials when the microbial leakage is evaluated.

The minimum and maximum periods (days) in which microbial leakage occurred and the mean rank of comparison of the test materials are shown in Tables 1 and 2. All positive control teeth showed microbial leakage and none of the negative controls leaked. Regards thermoplasticized gutta-percha, no statistically significant differences between this and the other three groups were observed.

DISCUSSION

An ideal root perforation sealing material should be dimensionally stable, radiopaque, easy to be manipulated, atoxic, nonocarcinogenic, nongenotoxic, biocompatible

Fig. 1: Specimen showing apical portion of the teeth immersed in the culture medium, and the upper part mounted on the Eppendorf tube sealed with cianocrilate and nail varnish, the tube-tooth unit is mounted inside the rubber cover and placed inside the glass flask.

and, if possible, stimulate healing process.[20] Furthermore, this material should present adequate solubility in oral fluids, satisfactory working time and antimicrobial activity.[2,3,12,13] Different materials have been used, such as gutta-percha, zinc oxide-eugenol, IRM, SuperEBA, glass ionomer, composites or MTA, among others.[5-10] Regarding the sealing capacity, have been evaluated by means of microbial filtration model used with slight modifications to control all variables as much as possible.[9,10,17,18]

It has been questioned the clinical relevance of marginal leakage studies using dye solutions[21] or coronal leakage assessed by microbial filtration, with the argument that bacterial penetration *in vitro* may not reflect the real conditions in mouth, by the absence of salivary enzymes, chewing forces or temperature changes, from observations in endodontically treated teeth exposed to the oral environment for extended periods.[22] Any *in vitro* methodology must consider limitations of clinic correlation from its results.[8-10] With respect to the methodology in this study, it should be pointed out that root-end filling materials are not exposed to temperature changes, masticatory forces or salivary enzymes. Microbial infiltration is a good indicator for these materials in terms of their capacity to block the leakage of microorganisms from the canal to the periapical tissues,[7] which is one of the requirements for these materials. However, any clinical extrapolation should be made with reserves. A methodology that resembled the clinical conditions was developed in order to control the variables through several methods: teeth were standardized in length; irrigation solutions (NaOCl, EDTA and saline) are routine used in clinic and retro-cavities had 3 mm deep; apical sealing except root-end cavity was made with a double layer of cyanoacrylate; the tube-teeth junction was sealed with a layer of epoxy resin, and a layer of nail varnish after the procedures described, to ensure a proper seal and avoid false positives.

The microorganisms used in this study have different morphological characteristics (cocci and rods), staining (Gram positive and negative) and respiratory (microaerophilic and anaerobic), and their selection was based on microorganisms evaluated in other studies;[23,24] the culture medium supports the nutritional requirements of used microorganisms.[23,25] The substitution of the microbial mixture every 7 days, and the determination of their viability is important to have always live microorganisms.[23]

The results obtained with ProRoot® MTA or Super EBA® show that both materials had microbial filtration in some specimens during the time period studied. The minimum time interval for microbial filtration was 41 and 28 days, respectively, without significant difference. In comparative studies on retrograde sealing ability evaluated by dyes, infiltration of endotoxin, proteins or microorganisms, MTA have shown similar[8,11,26,27] or better results[27,28] than amalgam, SuperEBA or IRM, even in blood presence.[29] Besides good sealing ability shown in previous studies,[11,16] in this study the results may be in part due to the antibacterial activity of Super EBA and MTA. In the case of SuperEBA attributed to eugenol, whereas MTA to its high pH by release of hydroxyl ions.[23]

Furthermore, the material that showed biggest leakage was Resilon. This could be explained because this material can not withstand long term exposure to bacteria, which may be caused by material contraction during polymerization and lack of adhesion to dentin, which can cause defects and gaps at the interface with dentin.[30] Pasqualini et al[31] pointed that Resilon can suffer biodegradation when is contacted with bacteria, due to enzymatic hydrolysis by bacterial lipases, which can break the ester linkages of the resin.

It is essential to evaluate the physical and chemical properties of root-end filling materials, as well as its biological response, before their clinical use.[14] An ideal root-end filling material must have good physical, chemical and antimicrobial properties besides be biocompatible.[5,7-9,12,15] It must be pointed that *in vitro* results should not be directly extrapolated to clinical conditions. Further research is required to support a broader clinical application of findings of this study.

CONCLUSION

Under the tested conditions, it was possible to observe that all the tested materials showed microbial leakage. Root-end fillings with Super-EBA or MTA had the lowest bacterial filtration, followed by those made with gutta-percha and AH Plus. The group that shows higher bacterial filtration was RealSeal.

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