Endodontic sealers: Intratubular penetration and permeability to *Enterococcus faecalis*

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ABSTRACT

Aim: Evaluate *in vitro* the intratubular penetration and permeability of endodontic sealers in teeth contaminated with *Enterococcus faecalis*.

Materials and Methods: Human canines were filled with AHPlus®, Endo CPM-sealer® or EndoRez® sealers. To evaluate permeability, the coronary portion of each tooth was contaminated with *E. faecalis*, then the apical portion was immersed in brain heart infusion (BHI) broth, and medium turbidity was observed for thirty days. Scanning electron microscope (SEM) was used to evaluate the intratubular penetration of each sealer at the cervical, middle, and apical thirds of the tooth.

Results: Only one tooth from the Endo CPM-sealer® group presented broth contamination. EndoRez® showed increased intratubular penetration compared to AHPlus® and Endo CPM-sealer®.

Conclusions: Endo CPM-sealer® showed greater permeability to *E. faecalis* and EndoRez® showed increased intratubular penetration.

Key words: Endodontic sealers, *Enterococcus faecalis*, intratubular sealing

The purpose of root canal filling is to maintain disinfection after chemical surgical preparation, promoting tridimensional sealing in order to avoid the penetration of periapical fluids, and the persistence of injuries. Even after correct root canal preparation, bacteria, such as *Enterococcus faecalis*, may remain within the interior of the dentinal tubules, leading to reinfection.[1-5]

The sealer must present good adhesiveness and leakage resistance to promote improved sealing, thus maintaining the bacteria inactive. When the sealer presents good leakage resistance properties, it can penetrate into the dentinal tubules; therefore, promoting better blockage. Greater adhesiveness promotes diminished displacement of the sealer into the root canal surroundings, providing greater obturation stability.[6-11] Numerous sealers were introduced to the market offering improved properties. Endo CPM sealer®, basically composed of MTA, is one of these materials and has been used in retrograde filling or perforation repair.

The aim of this study was to evaluate *in vitro* the intratubular penetration and permeability of the endodontic sealers AHPlus®, EndoRez®, and Endo CPM-sealer® to *E. faecalis* by microbiological analysis and scanning electronic microscopy (SEM).

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MATERIALS AND METHODS

The procedures of this work were conducted in accordance with the ethical standards of the committee responsible for ethics in research. Human canines (n = 34) were obtained from the tooth bank of the Dental Department and were hydrated in distilled water during the surgical preparation steps, which were performed by only one operator.

After accessing the pulp chamber, the canals were prepared by the step-back technique using Kerr file numbers 15 to 40 (Maillefer®, Ballaigues, Swiss) and irrigation with 5 ml of 1% sodium hypochlorite (Rioquímica®, São José do Rio Preto, SP, Brazil) and 5 ml of 17% EDTA-T (Manipulation pharmaceutics Resmer Vieira, Maringá, PR, Brazil) at every file change. Debridement of the apical foramen was performed using file number 15 K (Maillefer®).

Obturation of the root canals was performed by the lateral active condensation technique with endodontic sealers EndoRez® (Ultradent Products, South Jordan, UT, USA), Endo CPM-sealer® (Laboratório Egeo SRL, Argentina) and AHPlus® (Dentsply, Detrey, Konstanz, Germany). The setting times of each filling material respect the

recommendation of the manufacturer.

In order to analyze permeability to *E. faecalis* (ATCC 29212) from the pulp chamber to the apical foramen, the dental elements were divided into five experimental groups: G1, AH-plus® (n = 10); G2, Endo CPM-sealer® (n = 10); G3, EndoRez® (n = 10); G4, two teeth that were submitted to chemical surgery preparation, but were not obturated, serving as positive controls; and G5, two teeth that were not treated, serving as negative controls.

Three coatings of nail polish (Colorama®, São Paulo, SP, Brazil) were applied to the coronary and root portions of the teeth, except for the apical foramens, and then the teeth were placed in a rubber device (Tribico®, Lillo by Gerber, Rio de Janeiro, RJ, Brazil), maintaining the coronary portion inside the internal part of this rubber device and the root portion on the external part of the same. Sealing was performed using cyanoacrylate ester (Adesivo Instantâneo Universal, Super Bonder - Loctite®, São Paulo, SP, Brazil) and adhesive for Rigid PVC tubes and connections (Tigre®, Joinville, SC, Brazil). The drying time of the materials was respected: Nail polish (1 h each coating); cyanoacrylate ester (1 h) and adhesive for Rigid PVC tube and connections (12 h).[12]

The rubber/tooth device was constructed and cyanoacrylate ester was applied to fix the device to an acrylic bottle (baby's bottle HT clean 70 ml - KUKA®, São Paulo, SP, Brazil) filled with 20 to 30 ml of culture medium (BHI broth, Brain Heart Infusion, Difco, Detroit, MI, USA). The distance between the root apex and the culture medium was maintained at 5 mm. The acrylic bottle top was fixed to the screw on part of the same with cyanoacrylate ester.

The tooth/rubber tube connection and bottle/device were sterilized by gamma radiation (cobalt - 60) to 25 KGy (Irradiador Nordion, Canada, JS 9600) at the Brazilian Radiation Company (Embrarad, Cotia, SP, Brazil).

After this procedure, 10 μl of 24 h of *E. faecalis* broth was inoculated into the pulp chamber and incubated (Forma Scientific CO_2 Water-Jacketed Incubator) at 37°C in 5% CO_2. After every 72 hours, the broth remaining inside the coronary portion was removed and replaced with 10 μL of sterilized BHI broth.

The readings were performed daily throughout the 30-day experimental period. Whenever BHI broth turbidity was verified, a smear was performed using the Gram technique to identify the presence of the microorganism. The purity of the pulp chamber culture was determined weekly by BHI agar medium and the Gram technique.

In order to evaluate the intratubular penetration of endodontic sealers, teeth which permitted the permeation of *E. faecalis* during the experimental period (Endo CPM-sealer®) and another tooth from each group, which did not permitted the permeation of *E. faecalis* (AHPlus® and EndoRez®) was selected for analysis by scanning electronic microscopy (SEM) at the Aerospace Technical Center (CTA, of São José dos Campos, SP, Brazil).

Next the selected teeth were longitudinally cleaved (buccolingual plane) and two crevices were cut into them using a steel disc (KG Sorensen®): One device at the mesial face and the other at the distal face.

Metallization of the samples was conducted with a Denton Vacuum® machine, *Sputtering* system, for 3 min (300 Ã, 99.9999% gold). After this procedure, the samples were placed in a PD3 dehumidifier for 12 hours.

Two images of each root region (cervical, medium and apical) were obtained, totaling 36 images ×1000 magnification was used at the dentine interface and at the endodontic sealer.

The images were evaluated by six Endodontic specialists properly calibrated regarding the use of the Fotoscore Program.[13] The scores used to evaluate the intratubular penetration of the endodontic sealers were as follows: 2 (none or low penetration), 3 (regular penetration), and 4 (good penetration).

Comparison of the intratubular penetration of the sealers was performed by two-way ANOVA with the significance level set at 5%. The same statistical analysis was used to evaluate and compare the intratubular penetration of each sealer at each root region (cervical, middle and apical).

RESULTS

The positive control group (G4) presented contamination (culture medium turbidity) within the first 24 hours, and the negative control group (G5) presented no culture medium turbidity throughout the entire experimental period (30 days).

After 16 days, only one sample (10%) from G2 (Endo CPM-sealer®) permitted the permeation of *E. faecalis*. G1 (AHPlus®) and G3 (EndoRez®) showed no culture medium turbidity throughout the entire experimental period.

The EndoRez® sealer showed statistically significant increased penetration when compared to Endo CPM-sealer® ($P < 0.0001$) and AHPlus® ($P < 0.0001$). Endo CPM-sealer® and AHPlus® presented similar results ($P = 1$) [Figure 1].

Table 1 presents the mean and the standard deviation of the scores for intratubular penetration for each endodontic sealer and Table 2 presents the statistical correlation between the mean scores of the differents regions (cervical, middle and apical).

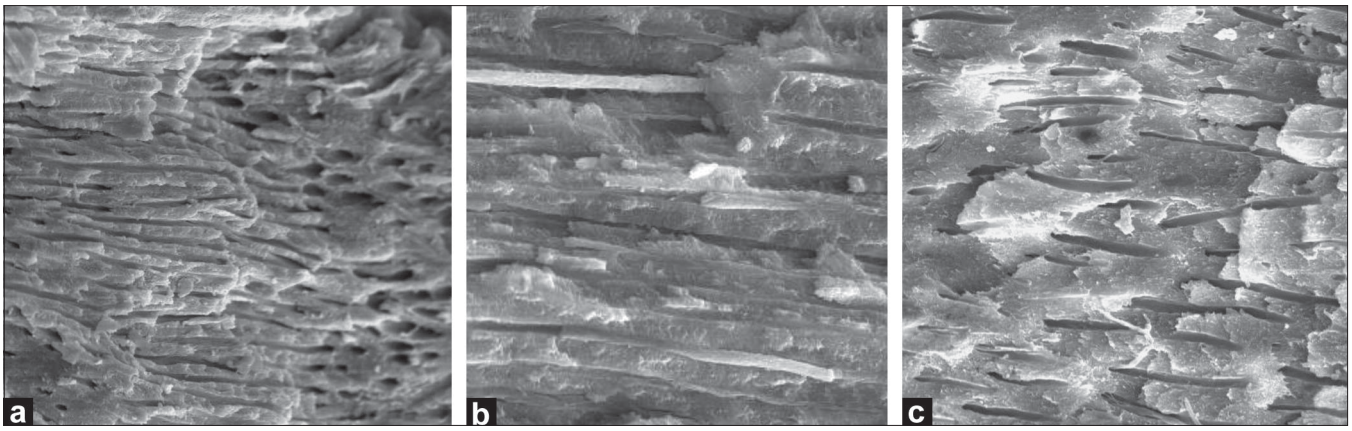

Figure 1: (a) Middle third with Endo CPM-sealer®: Low intratubular penetration, (b) Cervical third with EndoRez®: Good intratubular penetration, and (c) Apical third with AHPlus®: Regular intratubular penetration (×1000 magnification)

Table 1: Mean (μ) and standard deviation (σ) of scores for intratubular penetration of each endodontic sealer (×1000 magnification)

Sealer	Region	Resolution ×1000	
		μ	σ
Endo CPM-sealer®	C	2.16	0.38
	M	2.66	0.88
	A	2.66	0.65
AH-Plus®	C	2.75	0.75
	M	2.08	0.28
	A	2.66	0.49
Endo Rez®	C	3.08	0.66
	M	3.58	0.51
	A	3.75	0.45

C = Cervical; M = Middle; A = Apical

DISCUSSION

The persistence of microorganisms in root canals after chemical surgical preparation, even after using irrigating solutions and intracanal measuring, is well described in the literature.[11,14,15] *E. faecalis* was the microorganism of choice for the present study, because it is generally associated with endodontic treatment failure and the persistence of periapical injury.[1,2,5]

Endodontic infections caused by *E. faecalis* are not easy to treat due to the difficulty of eliminating this microorganism from the root canal system. *E. faecalis* shows resistance to most of the chemical agents used in endodontic therapy and seems to be one of the few pathogens that resist the antibacterial effects of calcium hydroxide.[5,11,14,15] *E. faecalis* pathogenicity in periapical tissue is related to its ability to form biofilm.[1,2]

Endodontic sealers are important in root canal obturation, because they complete the spaces that are not filled by gutta-percha, thus avoiding periapical exudates, making it difficult for resistant microorganisms to survive and preventing them or their products from reaching the apical region. The use of endodontic sealers associated with gutta percha became indispensable for proper sealing of the root canal system, improving the final quality of the treatment.[6,8,10,16]

In this study, although AHPlus® did not present the best result for intratubular penetration throughout the entire experimental period (30 days) it was able to prevent the permeation of *E. faecalis*, which suggests it possesses good apical margin sealing properties. AH-Plus® sealer showed increased intratubular penetration in the cervical and apical thirds while the Endo-Rez® sealer presented increased penetration in the apical and middle thirds. These results differ from those obtained by Sevimay and Kalayci[4] who found better adaptation of AH-Plus® and EndoRez® sealers in the cervical and middle thirds.

Since EndoRez® is a resinous sealer, good adhesiveness to the dental walls and leakage into the root canal system, providing adequate sealing of the root canals, were expected. This sealing was confirmed by the microbiological test, in which the sealer was able to prevent the permeation of *E. faecalis* throughout the entire experimental period.

Different results were obtained by Bouillaguet *et al.*[17] who observed greater micro leakage when using EndoRez®. Differences concerning these results may be related to the use of staining rather than the use of bacteria or it could be justified by the presence of humidity during the root canal obturation, leading to failure in sealer hardening. For this reason, when choosing EndoRez® as the obturation sealer, it is extremely important to maintain a dry environment, in order to promote correct sealer hardening.

Endo CPM-sealer® has a similar formulation to MTA (Mineral Trioxide Aggregate). Due to this similarity in composition and the successful clinical and laboratorial results attributed to MTA, Endo CPM-sealer® was used in this research as a root canal obturator material. Contamination was verified in 10% of the teeth with the use of this sealer; furthermore, it was the sealer which presented the lowest intratubular penetration.

Table 2: Statistical correlation between the means of each region (cervical, middle and apical) and the sealer evaluated at a resolution of ×1000 magnification

Sealer	Region	Endo CPM - sealer®			AHPlus®			EndoRez®		
		C	M	A	C	M	A	C	M	A
EndoCPM - sealer®	C				0.02			0.01		
	M					0.02			0.01	
	A						1.00			0.01
AHPlus®	C	0.02						0.18		
	M		0.02						0.01	
	A			1.00						0.01
EndoRez®	C	0.01			0.18					
	M		0.01			0.01				
	A			0.01			0.01			

C = Cervical; M = Middle; A = Apical; a statistically significant difference was considering when $P < 0.05$

A similar result was also obtained by Okada *et al.*[18] who verified greater apical percolation of Endo CPM sealer® compared to AH-Plus®.

In conclusion, EndoRez® sealer showed better sealing ability of the root canal system, good penetration in dental tubules (MEV) and did not permit the permeation of *E. faecalis* during the experimental period. AHPlus® did not present the best result for intratubular penetration, but it was able to prevent the permeation of *E. faecalis*.

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