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Comparison of bacterial leakage between Epiphany and AH Plus sealers using single cone and Tagger's hybrid obturation techniques

Patrícia de Almeida Rodrigues da Silva e Souza[a], Priscilla Rocha Affonso[a], Antônia Benedita Rodrigues Vieira[a], Ana Claudia Braga Amoras-Alves[a], Talita Tartari[a], Mario Honorato da Silva e Souza Junior[a]

[a] School of Dentistry, Federal University of Pará, Belém, PA, Brazil.

Abstract

Objective: To evaluate and compare, by means of bacterial infiltration, the quality of sealing obtained by Tagger's hybrid (TH) and Single Cone (SC) techniques, in association with AH Plus/Gutta-percha (AH) and Epiphany/Resilon (ER).

Methods: Palatal roots of 70 maxillary molars were instrumented and divided randomly into six groups: G1, TH/AH; G2, SC/AH; G3, TH/ER; G4, SC/ER; G5, negative control; G6, positive control. The roots were sterilized and monitored for 56 days to detect bacterial leakage using *Enterococcus faecalis*. The data were subjected to Binomial test ($\alpha<0.05$) to compare the infiltration among groups and Kruskal-Wallis test ($\alpha<0.05$) to analyze the infiltration as a function of time.

Results: All positive controls leaked within 24 h; none of negative controls leaked. There was no influence of time on leakage. Individual comparisons showed that there were no influences of the techniques and filling materials on quality of root canal sealing. Significant difference was found only between G1 and G4 ($p=0.0229$), the G1 showed a lesser and G2 the higher quantity of infiltration.
Conclusion: It could be concluded that none of the groups was able to prevent bacterial leakage and the lowest ability to prevent infiltration was obtained when applied SC/ER to filling the canal.

Key words: Bacteria; Dental materials; Endodontic; Root canal obturation; Dental leakage.

Comparação da infiltração bacteriana entre os cimentos Epiphany e AH Plus usando as técnicas de obturação de cone único e Híbrida de Tagger

Resumo

Objetivo: Avaliar e comparar, por meio da infiltração bacteriana, a qualidade de selamento obtido pelas técnicas híbrida de Tagger (TH) e do cone único (SC) associadas com AH Plus/Guta-percha (AH) e Epiphany/Resilon (ER) .

Métodos: Raízes palatinas de 70 molares superiores foram instrumentadas e divididas aleatoriamente em seis grupos: G1, TH/AH; G2, SC/AH; G3, TH/ER; G4, SC/ER; G5, controle negativo; G6, controle positivo. As raízes foram esterilizadas e monitoradas durante 56 dias para detectar a infiltração bacteriana utilizando *Enterococcus faecalis*. Os dados foram submetidos a teste Binomial ($\alpha<0,05$) ao comparar a infiltração entre grupos e Kruskal-Wallis ($\alpha<0,05$) para analisar a infiltração, como uma função do tempo.

Resultados: Todos os controles positivos infiltraram em 24 horas. Nenhum dos controles negativos infiltrou. Não houve influência do tempo na infiltração. Comparações individuais mostraram não haver influencia das técnicas e materiais de obturação na qualidade de selamento do canal radicular. Houve diferença significativa apenas entre G1 e G4 ($P=0,0229$), o G1 mostrou o menor e G4 a maior quantidade de infiltração.

Conclusão: Nenhum dos grupos foi capaz de evitar a infiltração bacteriana e a menor capacidade de prevenir a infiltração foi obtida quando utilizados de SC/ER para obturar o canal.

Palavras-chave: Bactérias, materiais dentários, endodontia, obturação do canal radicular, infiltração dentária.

Correspondence:
Patricia de Almeida Rodrigues da Silva e Souza
patsilesouza@uol.com.br

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Introduction

The presence of microorganisms in root canal systems is directly associated with the development of periapical disease. Successful treatments depend on the correct chemo-mechanical preparation followed by obturation and coronal restoration. These proceedings may prevent the failure of root canal treatment due to the persistence of microorganisms or secondary intraradicular infection [1]

Regarding filling materials, it is expected that they are able to seal the pulp canal space, in order to prevent the entry of tissue fluids, stimulate the apical repair, and that they also have an adequate biocompatibility to allow deposition of sealing cement to achieve biological seal [2]

One of the most widely used materials for root canal filling is gutta-percha in association with endodontic sealers. A great number of endodontic filling materials, in combination with different filling techniques, have been suggested every year to replace the technique of cold lateral condensation. This technique has been regarded as having some disadvantages such as lack of homogeneity of the gutta-percha mass, a high percentage of sealer in the apical portion of the canal and poor adaptation to the root canals walls [3]. Moreover, the strength required to manipulate the spreader may increase the risk of dentinal damage and vertical root fracture [4].

Alternative techniques allowing better adaptation to canal walls and a higher degree of homogeneity have been suggested [5,6]. The single cone technique (SC) consists of selecting a single gutta-percha cone matching the exact size and conicity of canals prepared with rotary instruments. The use of tapered cones with sealer could result in better adaptation to the root canal walls, without the requirement for accessory cones, being quicker than cold lateral condensation [3]. Therefore, the amount of sealer should be kept to a minimum, with only a thin layer between gutta-percha and the wall of the canal. However, the volume of root canal sealer required for the technique of single cone is larger than the volume used for most compaction techniques [7].

Thermoplastic techniques offer a substantial potential for obturation, which is equivalent or better than lateral condensation and less dependent on the canal shape if compared to the technique of condensation [2]. One of these techniques was Tagger's hybrid (TH) technique in which thermomechanical compaction is used after the lateral compaction of the apical third of the root canal [8]. The author proposed a modification of the original technique mentioned by John McSpadden in order to avoid extrusion of filling material toward the apical third.

The Resilon (Petron Clinical Technologies, Wallingford, CT, USA) is a thermoplastic synthetic polymer-based material for root canal filling. It performs like gutta-percha, has the same handling properties and the procedure for retreatment purposes may be the same as that of gutta-percha. This material is used with dual-curin resin-based sealer called Epiphany (Petron Clinical Technologies, Wallingford, CT, USA), which can be used with both warm and cold gutta-percha obturation techniques. Some researchers suppose that the result in a bond between the dentin and the obturation material creates a superior sealing compared to gutta-percha [9,10].

The AH Plus (Dentsply/De Trey, Konstanz, Germany) is a root canal sealer based on epoxide-amine resin, which has low solubility, adhesive characteristics, adequate flow, biocompatibility, antimicrobial activity and low shrinkage. No statistical difference in quality of root canal obturation with Resilon/Epiphany, SC, warm lateral condensation and System B was observed for some studies [5,11,12]. However, another study revealed significant difference between AH Plus, Resilon/ Epiphany, and Gutta-percha [13].

Both TH technique and the technique of SC provide a greater amount of solid filling material reducing the amount of root canal sealer present in the obturation, which could result in better quality of sealing. The aim of this study was to compare, by means of bacterial infiltration, the quality of sealing obtained by TH and SC technique, in association with AH Plus/Gutta-percha and Epiphany/Resilon.

METHODS

Preparation of specimens

In this study, seventy freshly extracted human palatal roots of upper molars were used, with straight canals and mature apices from subjects who signed an informed consent form under a protocol approved by the Committee on Ethics in Human Research of the University. Teeth were decoronated, leaving approximately 11 mm of root. They were subsequently stored in distillated water at 4°C until use.

Work length was determined by subtracting 1 mm from the length at which a #15 K-file (Dentsply-Maillefer, Bailagues, Switzerland) was visible at the foramen. The canals were prepared with torque control low speed engine with ProTaper system (Dentsply-Maillefer, Bailagues, Switzerland). The apical diameter for all specimens was standardized with a F5 instrument. After each file use, the canals were irrigated with 5 mL of 5% NaOCl (Biodinâmica Química e Farmacêutica Ltda., Ibiporã, PR, Brazil). The specimens received a final flush with 2 mL of 17% ethylenediaminetetraacetic acid (EDTA; Fórmula & Ação, São Paulo, SP, Brazil) for three minutes, to remove the smear layer; canals were dried with sterile paper points (Dentsply-Maillefer, Bailagues, Switzerland).

Root canal filling

After instrumentation, the roots were randomly divided into four test groups (n=15/experimental group) and two control groups (n=5/control group) and filled as follows:

- Group 1 (G1) – TH/AH: Tagger's hybrid technique, using a F5 ProTaper matching gutta-percha cone (Dentsply-Maillefer, Bailagues, Switzerland), extra fine accessory points (Dentsply-Maillefer, Bailagues, Switzerland) and AH Plus sealer.
- Group 2 (G2) – SC/AH: Single cone technique using a F5 ProTaper matching gutta-percha cone and AH Plus sealer.

- Group 3 (G3) – TH/ER: Tagger's hybrid technique, using a Resilon cone, extra fine accessory points (Pentron Clinical Tecnologies, Wallinford, CT, USA) and Epiphany sealer.
- Group 4 (G4) – SC/ER: Single cone using a Resilon cone and Epiphany sealer.
- Group 5 (G5) – Negative control: Teeth were cleaned, shaped and obturated similarly to the group TH/AH, and two coats of clear nail polish covered 100% of the root surface.
- Group 6 (G6) – Positive control: Teeth were obturated with gutta-percha single cone without sealer. Radiographs were taken to observe the quality of final obturation of all groups.

Bacterial leakage test

The model used in this study was based on a model previously described [5]. Briefly, the tapered ends of 1.5 mL polypropylene centrifuge tubes (Axygen Scientific, Union City, CA, USA) were cut and the obturated root was inserted with the apex pointing down. The junction between each tube and root was sealed with cyanoacrylate to prevent leakage of the connection. Two coats of clear nail polish were applied to all extruded root surfaces to the level of 2 mm from the apex. The entire device was then sterilized with ethylene oxide gas. After sterilization, the whole system was incubated at 37ºC for three days to ensure no contamination occurring before inoculation. One specimen from Group 1 was discarded due to this contamination. Each device was placed into culture tubes so that the apical part of each root was immersed in BHI (Brain Heart Infusion) broth. The bacterial suspension of the *E. faecalis* ATCC 29212 culture (Adolf Lutz Institute, São Paulo, SP, Brazil) at 10^8 colony-forming units/mL grown in BHI was used to contaminate root canals. A standard curve of *E. faecalis* cell numbers versus optical density was formed and used to determine the culture dosage necessary for inoculation. Each canal was aseptically inoculated with 0.1 mL of *E. faecalis* culture and all samples were incubated in a humid environment at 37ºC for eight weeks. The top of the devices were inoculated every seven days.

The bacterial leakage was determined by turbidity of the broth in the inferior chamber. At this point, 10 µL of the culture broth were subcultured onto blood agar to confirm the presence of *E. faecalis*.

The data were subjected to Binomial test ($\alpha<0.05$) to compare the infiltration among groups and Kruskal-Wallis test ($\alpha<0.05$) to analyze the infiltration as a function of time.

Results

The numbers of samples with bacterial leakage at the end of each week are listed in Table 1. The time of evaluation was 56 days and at the end of the observation the samples obturated by SC/ER had the highest number of infiltrated specimens (93,3%) following by TH/ER (86,7%), SC/AH (80%) and TH/AH (57,1%). No turbidity was observed in the negative group, indicating no leakage and confirming the efficiency of the bacterial infiltration device. All positive controls showed leakage within the first 24 hours.

Due to the difference in the amount of infiltrated specimens between groups, Binomial test was used to define whether the results were statistically significant (Table 2). The difference was detected only between groups TH/AH and SC/ER.

Time of bacterial infiltration was another hypothesis tested. There was no statistically significant difference between groups (Table 3).

Table 1. Number, percentage and total of samples infiltrated during the experimental period.

Groups	1WK	2WK	3WK	4WK	5WK	6WK	7WK	8WK	%	Total
TH/AH	4	1	1	2	0	0	0	0	57,1	8/14
SC/AH	2	2	1	1	1	2	1	2	80	12/15
TH/ER	5	2	2	2	1	1	0	0	86,7	13/15
SC/ER	2	1	2	3	2	2	1	1	93,3	14/15
Control $^-$	0	0	0	0	0	0	0	0	0	0/5
Control $^+$	5	0	0	0	0	0	0	0	100	5/5

Table 2. The P values (Binominal Test) of the comparison between experimental groups of bacterial infiltration.

Groups	HT/AH	SC/AH	HT/ER	SC/ER
TH/AH		0.1837	0.0755	0.0229*
SC/AH	–	–	0.6242	0.2827
TH/ER	–	–	–	0.5428
SC/ER	–	–	–	–

Discussion

The sealing ability of a root canal filling material is an important factor in preventing leakage of microorganisms and reinfection of root canal systems. The microleakage is a serious clinical problem and it may be responsible for unsuccessful treatments [14].

Gutta-percha combined with an endodontic sealer remains the most widely accepted and used obturation

Table 3. Number of samples infiltrated, first and last days in which there were infiltration and measures of central tendency in experimental groups.

Groups	Infiltrations	First day	Last day	Median	First quartile	Third quartile
TH/AH	8	1	24	7.00	3.25	17.75
SC/AH	12	1	51	26.5	8.75	38.75
TH/ER	13	1	36	8.00	3	23.00
SC/ER	14	1	52	22.50	15.25	34.50

technique. However, root canal sealers are subjected to problems, such as shrinkage and solubility, which provide an avenue for reinfection of the canal space [15,16]. Consequently, the objective of various filling techniques, as TH technique, is to maximize the amount of gutta-percha and minimize the amount of sealer. However, none of the sealers in conjunction with different techniques was able to avoid bacterial infiltration.

Single cone filling of root canals has been introduced to minimize the sealer component through the use of gutta-percha cones that closely match the geometry of nickel-titanium instrumentation systems. Some studies showed that good marginal adaptation was obtained with gutta-percha and epoxy resin-based sealer using either the system B or the single cone technique [17]. The results of this study showed that SC technique was not capable to prevent the bacterial infiltration and there were no advantages using the SC comparing with TH technique.

The adhesive capacity of Epiphany sealer is corroborated, in part, by the capacity of forming tags that protrude into the dentin tubules. However, this sealer shows high polymerization contraction leading to the formation of cracks between the obturation and the dentinal wall, and consequently microleakage [13,18). In this work, Resilon cone obturator was used with SC technique in group 4 and this group had the major number of infiltrations. It probably occurred because this cone does not have the same dimensions that gutta-percha of ProTaper system has, which may have resulted in a higher sealer line around the cone. The higher the sealer line is, the greater its disintegration and consequent marginal infiltration occurs [19,20]. Similar results were also observed by Sagsen et al [21].

In this study, the group 1 (TH/AH) resulted in a smaller number of infiltrated specimens. This is possibly because it makes use of plasticized material, which would promote greater fluidity and adaptation, in which the obturation "mass" would frame according to the format of the root canal [22].

With the purpose of reproducing the clinical condition, in which microorganisms and their products are frequently identified in endodontic lesions, the methodology of bacterial infiltration, in which micro-organisms are used as tracers of infiltration, was employed [5]. This is a technique that has a very similar protocol in most procedures, which makes it easy to standardize and compare results [5,6,9].

The influence of time in relation to the occurrence of infiltration was also tested; The TH/AH group was the one that showed a lesser quantity of infiltration, with a total of 42.9% of non-infiltrated specimens, but showed greater speed of infiltration. This fact can be related to failures occurred during the obturation of root canal systems, mainly regarding the formation of bubbles during the plasticizing of gutta-percha. Another relevant factor for this finding would be the final setting time of AH Plus, i.e., their total polymerization and hardening [23,24]. As this root canal sealer delays to set, this factor is associates with early occurrence of infiltration, because the fluid would not have difficulty in disintegrating the cement. However there was no statistically significant difference on time of infiltration between this and the other groups.

The results of the current study show that, independent of obturation technique, gutta-percha and root canal sealers cannot prevent the passage of bacteria to the periapical tissues. The results of the current study show that all samples obturated with gutta-percha and sealer without restoration (positive control group) exhibited extensive dye penetration. Therefore, after obturation of the root canal system, the occlusal access cavity should be properly sealed to improve the prognosis of endodontically treated teeth

The current study showed that, independent of obturation technique, gutta-percha and root canal sealers cannot prevent the passage of bacteria to the periapical tissues. Therefore, these results agree with the conclusions of other research that after obturation of the root canal system, the occlusal access cavity should be properly sealed to improve the prognosis of endodontically treated teeth [25].

Conclusions

Under the scope of this study, it was not possible to prevent the bacterial in none of the groups, and the lowest ability to prevent infiltration was obtained by applying SC/ER to filling the canal.

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