

Apical sealing ability comparison between GuttaFlow and AH Plus: *in vitro* bacterial and dye leakage

Comparação da capacidade de selamento apical entre os cimentos GuttaFlow e AH Plus: infiltração bacteriana e de corante in vitro

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Abstract

Objective – The success of the endodontic therapy demand a complete filling with maximum root canal system sealing, therefore the ideal filling should have a main and plentiful gutta-percha core associated to an endodontic sealer. In the effort to achieve an ideal filling material, arose the idea to incorporate gutta-percha powder and the sealer in a unique product, the GuttaFlow. **Methods** – Using 30 human teeth, the present study compared the marginal sealing capacity of the sealers GuttaFlow and AH Plus using the dual-chamber leakage model with *E. faecalis* during the experimental period of 60 days. Another leakage analysis was made by linear infiltration of the 1% methylene blue dye. **Results** – The results obtained from bacterial leakage showed no statistical difference under Fisher's exact test ($p = 0.500$). Similar results were founded from dye leakage that showed no statistical difference under ANOVA ($p = 0.575$) followed by t Test ($p = 0.1492$). **Conclusion** – These two sealers present similar behavior under booth marginal leakage methodologies.

Descriptors: Root canal obturation; Dental leakage/microbiology; *Enterococcus faecalis*; Coloring agents

Resumo

Objetivo – O sucesso do tratamento endodôntico requer uma obturação completa do sistema de canais radiculares, assim, a obturação ideal deve ter uma grande e principal massa de gutta-percha associada a um cimento endodôntico. Na tentativa de se alcançar este material obturador ideal, surgiu a idéia da união da gutta-percha em pó e do cimento em um só produto, GuttaFlow®. **Métodos** – Este estudo avaliou comparativamente a capacidade de selamento marginal do cimento endodôntico GuttaFlow e do cimento AH Plus, utilizando para tal o método de infiltração da bactéria *E. faecalis* em modelo experimental de dupla câmara pelo período de 60 dias complementado em seguida pelo método de infiltração do corante azul de metileno a 1% em 30 dentes humanos unirradiculares. **Resultados** – Os resultados obtidos pela metodologia de infiltração bacteriana foram analisados estatisticamente através do teste Exato de Fisher e demonstraram não haver diferença significativa entre os dois cimentos utilizados ($p = 0.500$); o mesmo aconteceu para a metodologia de infiltração de corante aonde verificou-se ausência de diferença estatisticamente significativa diante dos testes de análise de variância ANOVA ($p = 0.575$) e teste t ($p = 0.1492$). **Conclusão** – Os dois cimentos apresentam comportamento semelhante no que diz respeito à capacidade de selamento marginal frente às duas metodologias.

Descritores: Obturação do canal radicular; Infiltração dentária/microbiologia; *Enterococcus faecalis*; Agentes corantes

Introduction

The sealing of the root canal system is an important factor in the prevention of new infection caused by microorganisms or their by-products¹. To achieve this perfect marginal sealing, many techniques and materials are used. However, the technique most frequently recommended is the use of a large mass of solid and inert material (gutta-percha) associated with an endodontic sealer¹ applied using the lateral-condensation obturation method.

Among the current resin-based sealers, the AH Plus epoxy-amine resin-based sealer (Dentsply Maillefer, Ballaigues, Switzerland) is the most commonly used because of its good physical and chemical properties and good sealing ability²⁻⁵. However, with the aim of improving the marginal sealing properties of endodontic sealers, new sealers have been developed.

Due to a desire to obtain an ideal filling material, GuttaFlow (Coltène Whaledent, Altstätten, Switzerland) has been introduced with the idea of combining a large amount of solid material (gutta-percha powder) and a polydimethylsiloxane-based sealer into the same product. This new product is biocompatible and its physical, chemical and sealing properties make it suitable to this task, since it is similar to its predecessor³⁻¹⁰. But recent studies have shown different results for its sealing capacity^{5,11-15}.

To study the sealing property of new filling materials and techniques, several methods have been used: dye¹⁴, bacteria and their by-product^{8,12}, fluid transport^{5-6,9,11,13} and glucose penetration¹⁵. Ho-

wever, in order to establish coherent, trustworthy and reproducible methods, it is important to make possible transfer the obtained results to the clinic reality⁶. Therefore, the use of biological markers, such as bacteria and their byproducts may be helpful to evaluate marginal infiltration.

Considering that there are few studies analyzing the sealing capacity of these new endodontic sealers and due to their different conclusions, the present study evaluated the marginal apical sealing capacity of GuttaFlow and AH Plus endodontic sealers using the bacterial and Methylene blue dye leakage methods.

Methods

The research protocol was approved by the School of Dentistry of University of São Paulo Ethic Research Committee. Thirty-four extracted upper anterior teeth (incisors and canines) were cleaned externally, hydrated and kept stored in a 1% timol solution until used.

The working length and consequently the limit of the filling was determined by subtracting 1mm from the length of each dental element. The teeth were instrumented manually with K files #15 and #20 (Dentsply Ind. Com. Ltda., Petrópolis- RJ, Brazil), followed by rotary instruments using the K3 VTVT sequence (SybronEndo Corp., Orange, CA, USA). Irrigation was performed using 5 ml of 1% sodium hypochlorite solution between each file followed by a final flush with 10 ml of 15% citric acid solution (Laboratório F&A Ltda, São Paulo, SP, Brazil) for smear layer removal. A final rinse with 10

ml of 1% sodium hypochlorite was performed to remove any trace of this demineralizing solution.

After the cleaning & shaping phase, the teeth were dried externally with absorbent paper and the roots were dried by aspiration using capillary tips (Ultradent Products Inc., South Jordan, UT, USA), followed by sterilized absorbent paper cones (Dentsply Ind. Com. Ltda.).

For the external coating layer, a FM finger (SybronEndo Corp.) spreader was introduced into each apical foramen and all teeth were coated with a fast polymerizing epoxy resin, Araldite (Henkel Ltda, São Bernardo do Campo-SP, Brazil).

The teeth were randomly distributed and filled with the sealers under evaluation:

Group I – GuttaFlow sealer employing the lateral condensation technique.

Group II – AH Plus sealer employing the lateral condensation technique.

The other 4 teeth were allocated to the control groups. Two of them were left without obturation (positive control) and two were totally coated, including the coronary access (negative control).

The filled teeth were fixed in polypropylene Eppendorf tubes which had their ends cut off and the interface tooth/tube was sealed with fast polymerizing epoxy resin (superior chamber). The resulting models were sterilized with Gamma radiation (Cobalt 60 gamma cell) at 25 KGy according to the recommendations of the CTR IPEN/USP (Radiation Technology Center – Energy and Nuclear Research Institute – University of São Paulo, São Paulo-SP, Brazil).

The eppendorf/tooth model was put into a glass bottle (inferior chamber) which had been previously sterilized by steam that contained 3 mL of EVA broth medium (BD Corp., Franklin Lakes, NJ, USA). The interfaces between superior and inferior chambers were sealed with polyvinylsiloxane material to avoid the evaporation of the EVA broth from the inferior chamber.

The bacterial infiltration procedures were conducted in a laminar flow environment where 50 µL of a bacterial culture of *Enterococcus faecalis* at a concentration of 1.5×10^9 UFC/mL (degree 5 of the McFarland scale – BioMerieux AS, Marea l’Etoile, France) was placed in the superior chamber (Eppendorf) together with 250 µL of the EVA culture medium.

In order to keep the bacteria viable during the 60-day experiment, 200µL of the solution from the superior chamber was removed every two days and replaced with 200 µL of a new EVA broth medium. The experimental assemblies were kept in a 37°C incubator during the experiment.

During the experiment, the bacteria were able to leak through and reach the inferior chamber turning the broth medium cloudy, making it possible to confirm their presence due to the visible percolation. Once the leakage was verified, the specimen was removed and the number of the tooth and the date were recorded on a worksheet.

After the bacterial leakage experiment had gone full term (60 days), all specimens were removed from the Eppendorf and the cervical access was sealed with epoxy resin, then they were immersed in 1% Methylene blue dye (Laboratório F&A Ltda.) for 24hs.

After this period, the samples were washed for 24hs, the coated layer was removed, each tooth was included in polyester resin (Releasol Ltda, São Paulo, SP, Brazil) and then each one was longitudinally sectioned through the root canal center using a low-speed water-cooled circular saw Labcut 1010 (Extac Corp., Enfield, CT, USA) at 350 rpm.

The analysis of the dye’s linear leakage was done by image projection using a Nikon Optical Comparator Profile Projector (Nikon Instruments Inc., Melville, NY, USA), where it was possible to measure the linear distance of the infiltration in millimeters.

Results

The results obtained using the bacterial leakage method (Table 1) were analyzed statistically using Fisher’s Exact Test and showed that there are no significant differences between the two sealers used

($p = 0.500$). The same results were obtained for the dye leakage method (Table 2) where the absence of a statistically significant difference was shown when the ANOVA test ($p = 0.575$) was used followed by the t test ($p = 0.4192$). The two sealers present a similar behavior in regards to their marginal sealing ability.

Table 1. Number of bacterial infiltrated teeth during the experimental period and the average of time, in days, for each sealer tested

Group	Bacterial leakage (Teeth)	Average (Days)
G1 – GuttaFlow	11/15 (73.33%)	23.27
G2 – AH Plus	10/15 (66.66%)	20.10

Table 2. Average linear infiltration in millimeters of the Methylene blue dye for each sealer tested

Group	Linear dye leakage (mm)
G1 – GuttaFlow	4.194 ± 3.332
G2 – AH Plus	5.195 ± 3.354

Discussion

Based on preliminary studies with its predecessor, RoekoSeal RSA^{3,5-9,12,16}, the new silicon-based sealer GuttaFlow presents promising physical, chemical and biological properties.

To evaluate its sealing capacity, many methods have been employed to analyze the new sealers, among these the following have been used: dye¹⁴, bacteria and their byproduct^{8,12}, fluid transport^{5-6,9,11,13} and glucose penetration¹⁵.

Using these methods to compare the sealing abilities of the same sealers used in this study (AH Plus and GuttaFlow), several authors have shown that AH Plus is the material that has the best sealing capacity¹⁵, whereas others have indicated GuttaFlow as the best¹²⁻¹⁴.

Although it is not possible to do a direct relationship between the amount of infiltration and the endodontic treatment outcome⁶, the use of biological markers such as bacteria and their by products may help in the evaluation of marginal infiltration⁶, since this method has proven to be coherent, reproducible and similar to what happens *in vivo*^{9,12}.

The experimental model used in this study followed models previously described. It consisted of an upper chamber containing the marking agent (bacteria) and an inferior chamber containing the broth medium, separated by the coated specimen^{8,12}.

A recent study using the system described above showed lower infiltration levels for the polydimethylsiloxane-based sealer, GuttaFlow, when compared with AH Plus sealer and Pulp Canal Sealer for a period of nine weeks¹². Contrary to this though, when GuttaFlow sealer was compared to the AH Plus and Activ-GP sealers, the new GuttaFlow and Activ GP sealers showed higher infiltration (50% and 100%, respectively, over a period of 100 days), whereas AH Plus sealer proved to be more effective with 16.7% of the teeth being infected¹⁷.

Although cervical marginal leakage is more relevant¹⁸, this study evaluated apical percolation, since this part of the root canal system is still relatively vulnerable to bacterial infiltration. This failure results in the presence or persistence of intra-radicular infection that induces inflammatory and immunological responses in the periapical tissue.

Considering the fact that there are few studies evaluating the sealing capacity of this new endodontic sealer using bacteria³ as a leakage marker, the aim of this study was to evaluate the marginal apical sealing capability of GuttaFlow and AH Plus endodontic sealers using the dual-chamber leakage method and *Enterococcus faecalis* as the marker.

The results of this study, obtained from the bacterial infiltration method, showed a similar behavior ($p > 0.05$) between the GuttaFlow sealer (73.33% in 23.37 days on average) and the AH Plus sealer (66.66% in 20.10 days on average). Similar results were found using the linear dye leakage method, where the GuttaFlow sealer

(4.194mm ± 3.332) and the AH Plus sealer (5.195 ± 3.354) did not show any significant difference ($p > 0.05$).

These results are in agreement with recent study¹¹ which have analyzed the quality of the seal provided by AH Plus and GuttaFlow sealers using the fluid transport analysis method and several filling techniques. In these study, the authors had shown that the single cone filling technique used in conjunction with the GuttaFlow sealer produces similar results when compared to the vertical condensation technique of gutta-percha cones with AH Plus sealer¹¹. The same results were obtained when using the continuous waves of condensation technique associated with the AH Plus sealer⁵.

Although there are doubts regarding the filling capability of this filling material, which seems to be deficient because of the voids present in the filling mass¹⁹, its adequate marginal sealing capacity can be explained by the expansion property (0.16% at 25°C and 0.76% at 37 °C) of this polydimethylsiloxane-based sealer during polymerization²⁰.

Conclusion

Within the limitations of this study, we can conclude that there are no statistically significant differences between GuttaFlow and AH Plus sealers based on the bacterial and dye infiltration methods. Therefore, we can consider GuttaFlow sealer as being a promising endodontic sealer. However, further studies on its marginal sealing capacity are necessary.

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