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A comparative evaluation of antimicrobial efficacy and flow properties for Epiphany, Guttaflow and AH-Plus sealer

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Abstract

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Aim To test the antimicrobial efficacy and flow properties of Guttaflow, Epiphany sealer and AH-Plus sealer.

Methodology With the use of *Enterococcus faecalis ATCC 29212* as a test organism, both the agar diffusion test (ADT) and direct contact test (DCT) were performed. For DCT, sealers were mixed and placed over the bottom of sterile screw-capped test tubes. A 50μL bacterial suspension was placed on the tested material samples. Bacteria were allowed to directly come in contact with the sealers for 1 h at 37 °C in one group and for 24 h in the other group. The suspensions were then diluted and inoculated over blood agar plates, and bacterial colony counts were determined with the use of a digital colony counter. The data in both 1- and 24-h groups were individually analysed using repeated measures ANOVA. Kruskal Wallis tests were further used to obtain comparison between 1- and 24-h results for all three sealers. In the flow assay, the sealers were placed between two glass slides, and a weight of 500 g was placed on the top of the glass. The diameters of the formed discs were recorded.

Results For both the ADT and DCT tests, Epiphany and AH-Plus sealer reduced the bacterial counts significantly ($P = 0.000$). Epiphany produced a greater reduction in bacterial counts when compared to AH-Plus in both the tests ($P = 0.000$). Guttaflow paste failed to show any antibacterial activity in both ADT & DCT. According to the flow test, all root canal sealers flowed; Epiphany sealer had the maximum flow under the given conditions, followed by AH-Plus sealer and Guttaflow paste.

Conclusions Antimicrobial activity of the sealers was greatest for Epiphany followed by AH-Plus sealer and Guttaflow. Epiphany sealer had the maximum flow followed by AH-Plus sealer and Guttaflow.

Keywords: agar diffusion test, AH-Plus, direct contact test, *E. faecalis, Epiphany, Guttaflow.*

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Introduction

Microorganisms and their products are the main aetiological factors in dentinal, pulpal and periapical pathosis (Kakehashi *et al.* 1965, Brannstrom & Nordenvali 1978, Fabricus *et al.* 1982, Barnett *et al.* 1990, Sundqvist 1992). The central aim of root canal treatment is the elimination of bacteria from the infected root canal and prevention of subsequent reinfection. This is mainly achieved by thorough irrigation and biomechanical preparation of the root canal, followed by a canal filling that should seal the canal system from bacterial ingress from the oral cavity and periradicular tissues. Long-lasting sealing ability and adaptation to the root canal walls are one of the prime requisites for a root canal sealer.

The presence of bacteria in dentinal tubules and cementum even after treatment has been reported. (Dalton *et al.* 1998, Molander *et al.* 1999, Shuping

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et al. 2000, Chavez De Paz *et al.* 2003, Sundqvist & Figdor 2003). Microbial persistence and growth in dentinal tubules, lateral canals and apical ramifications have also been demonstrated (Love & Jenkinson 2002, Torabinejad *et al.* 2002). A well-adapted sealer will only hinder the release of bacteria entrapped within the root canal system. But, for eradication of the remaining microorganisms, particularly when pulpal necrosis and apical periodontitis are present, the choice of a sealer with substantial antimicrobial activity could play an important role (Spangberg *et al.* 1973).

Microorganisms infecting the root canal system might adhere superficially to the dentinal wall or penetrate into the dentinal tubules (Ando & Hoshino 1990, Peters *et al.* 2001). Superficially adhering bacteria are likely to be killed more easily than those shielded in the depths of dentinal tubules. These microorganisms inside the dentinal tubules might be challenged by antimicrobial components leaching from the sealer. Hence, testing of the antimicrobial efficacy of sealers should take into account these two effects based on the contact and diffusibility of the sealer.

Most laboratory studies use agar diffusion test (ADT) as the standard assay despite its acknowledged limitations (Tobias 1988). This method does not distinguish between microbiostatic and microbicidal properties of the material. The antimicrobial activity of the sealer indicated by this test is influenced by the solubility and diffusibility of the material in the medium. On the contrary, the direct contact test (DCT) (Weiss *et al.* 1996) measures the effect of direct and close contact between the organisms and the material, regardless of the solubility and diffusibility of the antimicrobial components of the sealer.

However, the desirable properties of sealers such as antimicrobial efficacy or providing a good seal will be less relevant if the sealer does not flow adequately into canal irregularities (Wolcott *et al.* 1997). The ability of a sealer to flow is important as it reflects its capacity to penetrate into small irregularities and ramifications of root canal system and dentinal tubules (Siqueira *et al.* 1995, Weis *et al.* 2004). Moreover, sealers that have antimicrobial efficacy as well as the ability to flow may aid in the elimination of microorganisms from the canal (Siqueira *et al.* 2000).

To achieve the properties of an ideal endodontic sealer, newer sealers are continually introduced in the market. Amongst them, Guttaflow, a new flowable root canal filling paste, is a cold flowable system that combines both the sealer and the gutta-percha in one product. The sealer is silicone-based polymethyl hydrogen siloxane as its main component. The powder consists of finely ground gutta-percha (0.9 μm). It has shown good homogeneity and adaptation to the root canal walls owing to its better flow properties (Elayouti *et al.* 2005). Another group of sealer that has gained popularity is the resin-based materials. The sealer Epiphany (Pentron clinical Technologies, Wallingford, CT, USA) is a dual curable resin composite sealer. Although the antimicrobial efficacy of AH-Plus (Dentsply, de Trey, Konstanz, Germany) has been tested previously, studies on Epiphany sealer and Guttaflow paste are limited. The hypothesis tested in this study is that Epiphany and Guttaflow had antimicrobial efficacy.

Therefore, this study was conducted to test the antimicrobial efficacy of AH-Plus sealer, Epiphany sealer and Guttaflow paste using both ADT and DCT. The flow rate of the same sealers was also tested at room temperature.

Materials and methods

Materials

AH-Plus sealer, resin-based Epiphany sealer and Guttaflow obturation paste were tested and compared (Table 1). The sealers were prepared in compliance with the manufacturer's recommendations. ADT and DCT were used to evaluate the antimicrobial activity. Both tests were undertaken under strict aseptic precautions in a laminar airflow cabinet (Kartos Int., Noida, India).

Agar diffusion test

The antimicrobial sensitivity test was performed on Mueller Hinton Agar (Himedia Laboratories, Mumbai, India) using the Kirby Bauer method (Washington *et al.* 2006). The surfaces of ten freshly prepared Muller Hinton agar plates were inoculated with 0.2 mL BHI broth culture of *Enterococcus faecalis ATCC 29212*. Three wells of 4 mm depth and 5 mm diameter were punched in each of the agar plates. The three freshly mixed sealers were then placed in the wells of each agar plate. The plates were then incubated at 37 °C for 24 h. The diameters of the zones of inhibition around each well were then measured in millimetres (mm). A mean diameter was determined for each of them under 2.5× magnification. Zones of inhibition were analysed statistically to assess antimicrobial activity of the tested sealers using repeated measures ANOVA test with $P = 0.05$ as the level of statistical significance.

Table 1 Composition of sealers

Sealer	Composition	Manufacturer
AH-Plus	Pasta A (epoxy): diglycidil-bisphenol-A-ether, calcium tungsten, zirconium oxide, aerosol, iron oxide Paste B (amina): amina-1-adamantane, *N*,*N*-dibenzyl-5-oxanonandiamine-1,9,TCD-di-amine, calcium tungsten, zirconium oxide, silicone oxide	Dentsply De Trey, Konstanz, Germany
Epiphany	UDMA, PEGDMA, EBPADMA, BISGMA resin, silane-treated barium borosilicate glasses, barium sulphate, silica, calcium hydroxide, bismuth oxychloride with amines, peroxide, photoinitiator, stabilizer, pigments	Pentron Clinical Technologies, Wallingford, CT, USA
Guttaflow	Paste A (sealer): poly-dimethyl polymethyl hydrogen siloxane, silicone oil, paraffin oil, zirconium dioxide and platin catalyst Paste B (powder): Guttapercha (0.9 μm), zinc oxide, barium sulphate and nanosilver particles (as a preservative)	Coltene Whaledent, DPI, Mumbai, India

Direct contact test

The DCT is based on turbidometric determination of bacterial growth in 96-well microtitre plates. The test performed to assess the antimicrobial properties of the endodontic sealers was a modification of the DCT described by Weiss *et al.* 1996. In brief, 50 mg of AH-Plus sealer, Epiphany sealer and Guttaflow paste were measured and placed into 20 sterile screw-capped test tubes of equal sizes for each sealer and spread over its base After the recommended working time of the sealers (approximately 15 min later), a 50 μL McFarland standard (1.5×10^8 CFU mL^{-1}) suspension of *E. faecalis ATCC 29212* was pipetted and spread over the sealers. The test tubes were incubated at 37 °C in a humid atmosphere. This allowed the liquid to evaporate and ensured direct contact between bacteria and test sealers.

The suspension of *E. faecalis* and sealers was allowed to be in contact for 1 h in one group and for 24 h in the other group for all the three sealers.

To determine the colony count of the suspension in both groups, the suspensions were diluted by adding 450 μL of sterile BHI broth to the screw-capped tubes. From each of these vials, 10 μL of suspension was drawn and spread over blood agar to determine the colony count with a digital colony counter (Serwell Instruments Inc., Bangalore, India).

A suspension of *E. faecalis ATCC 29212* without the sealer was taken as the control and subcultured after 1 and 24 h, and the colony count was determined. Colony counts of the three sealers in both the groups (1 and 24 h) were also determined in a similar manner. This indicated the antimicrobial efficacy of the three materials against *E. faecalis* at 1- and 24-h intervals, i.e. immediate and delayed antimicrobial efficacy. This data in both 1- and 24-h groups were individually analysed by using a repeated measures ANOVA test to find the difference in the antimicrobial efficacy of all three sealers at a given time interval. Kruskal Wallis test was further used to obtain comparison between 1- and 24-h results for all three sealers.

Flow test

The flow test was conducted at room temperature as described by Benatti *et al.* (1978) and Siqueira *et al.* (1995). Half a millilitre of each sealer was prepared and placed between two glass slabs, and a weight of 500 g was placed on top of the glass (weighing 20 g) for 1 min. Hence, the total weight acting on the specimen was 520 g. Ten samples of each sealer were used. The diameters of the formed disc were measured and analysed for statistical significance using a repeated measures ANOVA test.

Results

Agar diffusion test

The zones of bacterial growth inhibition obtained from the ADT in mm for each of the sealers are noted in Table 2. AH-Plus sealer had an average inhibition zone of 9.6 mm (Fig. 1a) and the Epiphany group the largest mean inhibition zone (18.1 mm) (Fig. 1b). Guttaflow group had no inhibition zone (Fig. 1c). There was a statistically significant difference between all the three groups ($P = 0.000$). Further post hoc analysis for groupwise comparisons showed that the Epiphany group exhibited significantly higher microbial inhibition ($P = 0.000$) than both AH-Plus sealer and Guttaflow. Guttaflow did not demonstrate any microbial inhibition.

Table 2 Mean diameters of zones of inhibition for the ADT, Colony counts of the three sealers per mL in 1- and 24-h groups of DCT and mean flow diameters for the three test sealers

Groups	ADT (diameter in mm)	DCT ×10^8 no. of organisms per mL		FLOW (diameter in mm)
		1 h	24 h	
Control	0	1.5	1.5	0
AH-Plus	9.60 ± 0.70	0.26 + 0.02	0.02 + 0.01	10.90 + 0.57
Epiphany	18.10 + 0.74	0.02 + 0.01	0.03 + 0.09	12.20 + 0.63
Guttaflow	0	1.5	1.5	8.90 + 0.74

ADT, agar diffusion test; DCT, direct contact test.

Direct contact test

The results of the DCT are shown in Table 2 and Fig. 2 for all three groups. AH-Plus and Epiphany had significantly lower bacterial counts when compared to the control group in both 1-h ($P = 0.000$) and 24-h groups ($P = 0.000$), indicating substantial antimicrobial efficacy of the two sealers. Amongst the three groups, Epiphany had the highest antimicrobial efficacy, which was significantly greater than both AH-Plus ($P = 0.000$) and Guttaflow group ($P = 0.000$). Guttaflow did not cause a reduction in bacterial counts at both the time intervals, indicating absence of antimicrobial affect.

On further statistical analysis of 1- and 24-h data, both AH-Plus and Epiphany groups produced a significant ($P < 0.05$) reduction in the bacterial growth on subculture at 1- and 24-h intervals. This further indicates the increased antimicrobial efficacy of both Epiphany and AH-Plus sealer with time. The Guttaflow group, however, failed to show any reduction in bacterial counts over this time period. This further confirmed the lack of antimicrobial efficacy of Guttaflow.

Flow test

The averages of the mean diameters of the discs are presented in Table 2. All root canal sealers flowed but there was a significant difference in the flow between all three sealers ($P = 0.000$). The Epiphany sealer had the greatest flow followed by AH-Plus resin sealer. Guttaflow flowed least.

Discussion

An ideal root canal sealer should have good antimicrobial activity and good flow. (Grossman 1976). The purpose of this study was to evaluate the antimicrobial efficacy of AH-Plus, Epiphany and Guttaflow

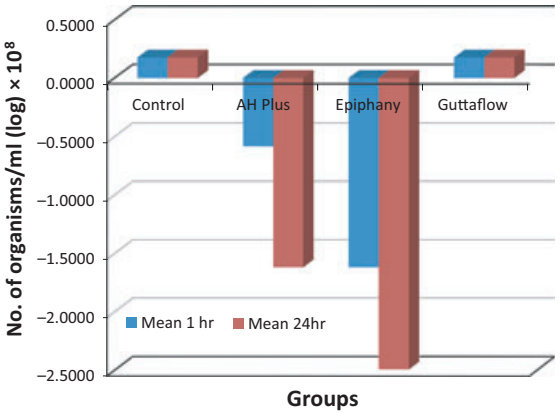

Figure 2 Graphical representation of log values of growth of colony counts of the three sealers by the direct contact test.

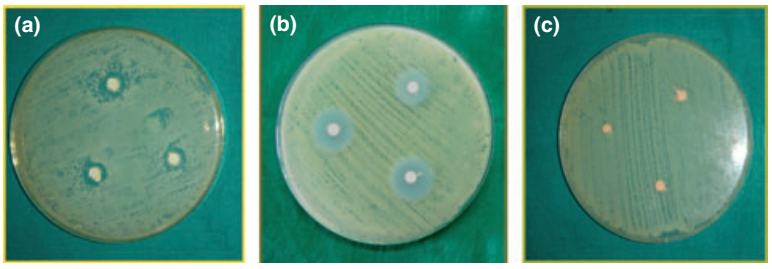

Figure 1 Zone of inhibition for AH-Plus sealer (1a), Epiphany sealer (1b) and Guttaflow (1c).

sealer against the facultative anaerobic bacteria *E. faecalis* and to compare the flow of these sealers.

Enterococcus faecalis was chosen as it is commonly found in infected root canals. It is most often isolated in retreatment cases of apical periodontitis (Roach *et al.* 2001). Its prevalence ranges from 24% to 77%. This finding can be explained by various virulence factors, including its ability to compete with other microorganisms, invade deeply into dentinal tubules and resist nutritional deprivation (Charles *et al.* 2006). Thus, antibacterial action against these bacteria is relevant to clinical practice. *faecalis* ATCC 29212 was used.

Antimicrobial efficacy was first assessed by an ADT and then with a DCT. The ADT measured the antimicrobial activity by determining the size of the zone of bacterial growth inhibition in agar formed around the specimen in a 24-h period.

The size of the zone of bacterial inhibition from an antimicrobial substance on a bacteriological culture medium depends upon: (i) toxicity of the substance towards the particular bacterium and (ii) the diffusibility of the substance in the test medium used. Thus, a less diffusible sealer would result in a smaller or no zone of inhibition. The diffusibility of the agent is a function of its hydrophilicity or hydrophobicity and the size and rate of release from the insoluble matrix in which it is bound (Barry & Thornsberry 1980). These variables are difficult to control and may vary from one material to another. Also, an important requirement for any filling material is its resistance to solubility and degradation when exposed to a host environment for a prolonged period. This basic requirement is contradictory to the requirement of solubility for testing the antimicrobial efficacy of materials by agar diffusion (Weiss *et al.* 1996).

Hence, to overcome this limitation of the ADT, a DCT was also performed. This assay relies on direct and close contact between the test organisms and the test material and is virtually independent of the diffusion and solubility properties of both the tested material and the media (Weiss *et al.* 1996).

Two assays measure different properties of the antibacterial components. ADT results indicate the activity of freshly mixed materials and the existence of diffusible components into aqueous milieu. Inclusion of ADT is important for comparative reasons with previous studies; DCT shows the activity of insoluble antibacterial components and can be used in standardized ageing studies. It is proposed that in the process of evaluating antibacterial properties of dental materials, in particular endodontic sealers, more than one assaying method should be used.

Guttaflow failed to show any antimicrobial effectiveness when tested by both methods. Similar results have been reported previously (Brzovic *et al.* 2007, Eldeniz & Ørstavik 2007, Mohammadi & Yazdizadeh 2007). The nanosilver component specially added in this system might have acted as a preservative, but it does not contribute towards antimicrobial efficacy, according to the present study.

AH-Plus sealer had greater antimicrobial activity than Guttaflow in both the ADT & DCT tests. Cobankara *et al.* (2004) also reported that AH-Plus is a more potent bacterial inhibitor than Roeko-Seal, a sealer similar in composition to Guttaflow. Other studies have also reported the antimicrobial activity of AH-Plus sealer (Kayaoglu *et al.* 2005, Eldeniz *et al.* 2006). The antimicrobial effect of the resin-based sealer AH-Plus may be related to bisphenol diglycidyl ether, which was previously identified as a mutagenic component of the resin-based sealer (Heil *et al.* 1996). In addition, it has been reported that the material releases formaldehyde during polymerization (Leonardo *et al.* 1999).

The Epiphany sealer had the greatest antimicrobial activity, both through ADT and DCT. Previous studies also support these findings (Bodrumlu & Semiz 2006, Brzovic *et al.* 2007). Bodrumlu & Semiz (2006) reported that Epiphany was more effective than AH 26 sealer, but its efficacy was less than Sealapex and Endomethasone that contain $Ca(OH)_2$ and zinc oxide, respectively. Brzovic *et al.* (2007) reported that Epiphany and IRM had greater antimicrobial activity than Guttaflow and Diaket. Eldeniz & Ørstavik (2007) reported that Epiphany was antibacterial, but it was lower than RC sealer, a eugenol-based sealer.

Kaplan *et al.* (1999) stated that eugenol and formaldehyde containing sealers have significant antimicrobial activity. Hence, it can be concluded that Epiphany may not be the sealer with highest antimicrobial activity. However, it possesses the potential for antimicrobial activity and is more effective than Guttaflow.

Antimicrobial efficacy was tested at 1- and 24-h intervals. The results indicated highest antimicrobial efficacy of Epiphany sealer at both time intervals, followed by AH-Plus sealer.

Another important property of sealers that was tested was flow of the sealers. This property allows the sealer to penetrate into irregularities, isthmi, fins and ramifications, which increases the likelihood of obtaining an adequate seal of the root canal system (Siqueira *et al.* 1995, Weis *et al.* 2004. Moreover, sealers that possess both antimicrobial properties and optimum flow ability

might theoretically eliminate microorganisms located in such confined areas of the root canal system. Data from the flow test showed that all sealers flowed; however, Epiphany sealer had superior flow followed by AH-Plus sealer and Guttaflow.

Conclusions

1. Antimicrobial activity of the sealers occurred in the following decreasing order: Epiphany>AH-Plus>Guttaflow.
2. Flow of the sealers occurred in the following decreasing order: Epiphany>AH-Plus>Guttaflow.

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