Antibacterial Activity of a New Endodontic Sealer against *Enterococcus faecalis*

Emre Bodrumlu, *DDS, PhD;* ***Mustafa Semiz,*** *MSc, PhD*

Contact Author

Dr. Bodrumlu
Email: *bodrumlu@omu.edu.tr*

ABSTRACT

Aim: The purpose of this study was to test a new root canal sealer (Epiphany) and 5 other root canal sealers (Diaket, Endomethasone, AH 26, Sealapex, Sultan) for their antimicrobial effect on *Enterococcus faecalis*.

Materials and Methods: The antimicrobial effect of 6 root canal sealers was tested by the agar diffusion method. The freshly mixed sealers were placed in prepared wells of agar plates inoculated with *E. faecalis*. All plates were incubated for 72 hours at 37°C under aerobic conditions, and zones of inhibition were measured at 24, 48 and 72 hours.

Results: All of the sealers caused bacterial growth inhibition. Their effectiveness, in descending order of antimicrobial activity, was as follows: Endomethasone, Sultan, Sealapex, Diaket, Epiphany and AH 26. Epiphany sealer had little effect on the tested microorganism. The effectiveness of the root canal sealers decreased marginally with greater duration of incubation.

Conclusions: The Epiphany root canal sealer offered no antibacterial advantage over the other sealers tested.

MeSH Key Words: *Enterococcus faecalis*/drug effects; materials testing; root canal filling materials/pharmacology

© *J Can Dent Assoc* 2006; 72(7):637
This article has been peer reviewed.

One of the key goals of endodontic therapy is complete obturation of the root canal system. The success of obturation is directly related to the elimination of microorganisms through mechanical cleaning and shaping, supplemented by antibacterial irrigants, adequate filling of the empty space, and use of antimicrobial dressings (with calcium hydroxide) between appointments, if necessary.[1,2] However, these procedures do not result in complete sterility of the root canal space.[3] Therefore, antimicrobial agents are added to root canal sealers to improve their antibacterial effect.[4,5]

Grossman[5] advocated that the ideal root canal filling material should be bacteriostatic. Sundqvist and others[2] recovered numerous species of anaerobic bacteria such as *Enterococcus faecalis*, *Streptococcus anginosus*, *Bacteroides gracilis* and *Fusobacterium nucleatum* after "failed" root canal therapy. Although *Enterococcus* spp. usually constitute a small proportion of the initial flora in the untreated root canal,[6] this genus is the most commonly recovered from the root canals of teeth with failed root treatment[2] and has also been implicated in persistent root canal infections.[7]

Table 1 Mean diameter of zones of growth inhibition with 6 root canal sealers

	Duration of incubation; mean diameter (SD) of inhibition zone (mm)[a]		
	24 h	48 h	72 h
Endomethasone	35.50 (1.29)	33.50 (1.29)	32.50 (1.73)
Sultan	31.25 (1.50)	30.50 (1.73)	28.25 (1.25)
Sealapex	24.00 (2.16)	22.00 (2.16)	21.75 (2.21)
Diaket	22.75 (2.06)	19.75 (1.70)	19.00 (1.41)
Epiphany	20.00 (0.81)	19.25 (0.95)	18.00 (1.63)
AH 26	14.75 (0.95)	14.25 (1.25)	13.75 (0.95)

[a]*Each value is the mean of 4 wells; SD = standard deviation*

The purposes of root canal sealers are to prevent recolonization by bacteria and recontamination of the canal system, to prevent the growth of residual bacteria in the root canal system[5] and to eliminate gaps between the core filling material and the canal walls. The agar diffusion method has been widely used to test the antimicrobial activity of root canal sealers.[3,8,9]

The objective of the present study was to test a new root canal sealer and 5 other root canal sealers for antimicrobial activity against *E. faecalis*, by measuring the diameter of zones of growth inhibition on the surface of agar plates.

Materials and Methods

Six root canal sealers were tested for antimicrobial activity against *E. faecalis*.

The bacteria were cultivated in solid media, and broth culture suspensions were prepared and adjusted to No. 1 McFarland standard (approximately 3×10^8 cells/mL). Aliquots of the suspension containing *E. faecalis* were spread on four 140-mm diameter Petri dishes containing Mueller-Hinton Agar medium (Merck, Darmstadt, Germany). Excess inoculum was removed with a pipette, and the inoculated plates were dried for 15 minutes at 37°C. Each plate was divided evenly into 6 sections. In each section of each plate, a well 5 mm in diameter was created with a sterile stainless steel cylinder.

The following 6 sealers were mixed according to the manufacturer's instructions: Diaket (3M ESPE AG, Seefeld, Germany), Sealapex (Kerr Italia S.p.A., Torino, Italy), AH 26 (Dentsply De Trey GmbH, Konstanz, Germany), Epiphany (Pentron Clinical Technologies, LLC, Wallingford, Conn.), Endomethasone (Spécialités Septodont, Saint-Maur-des-Fosses Cedex, France) and Sultan (Sultan Chemists Inc., Englewood, N.J.). A sample of each freshly mixed dental material was placed into wells in each section of the 4 plates. Each experiment was replicated 4 times.

All plates were incubated for 72 hours at 37°C under aerobic conditions, and zones of growth inhibition were measured at 24, 48 and 72 hours.

The diameter of the growth inhibition zones was analyzed by 2-way analysis of variance (ANOVA). The interaction of root canal sealer and duration of incubation was not statistically significant ($F = 0.651$, $p = 0.76$). The effects of each factor were analyzed by 1-way ANOVA, and the inhibition zones associated with each sealer and each duration of incubation were compared by Tukey's honestly significant difference (HSD) pairwise comparisons. All statistical analyses were performed with the SPSS statistical software package, and all results were evaluated at the 5% significance level.

Results

The mean diameters of zones of inhibition caused by the 6 root canal sealers are presented in **Table 1**. The 4 trials yielded consistent results.

All 6 root canal sealers caused zones of inhibition. However, the new root canal sealer, Epiphany, and the AH 26 sealer had little effect on the tested microorganism. The Endomethasone and Sultan sealers exhibited the largest inhibition zones (mean diameter about 33 mm). The Diaket and Sealapex sealers showed mild antimicrobial activity against *E. faecalis* and were more effective than the Epiphany and AH 26 sealers (**Table 2**).

The results after 48 and 72 hours showed that the effectiveness of the root canal sealers decreased slightly with time (**Table 3**).

Discussion

The persistence of bacteria in the root canal system often leads to failure of root canal treatment. Enterococci have been shown to survive in root canals as single organisms.[10] *E. faecalis*, which is often associated with persistent apical periodontitis, was chosen as the test organism for this study because it may be difficult to eliminate from root canals.[11] The antibacterial activity of root canal sealers against this facultatively anaerobic microorganism may assist in controlling infection, given the high prevalence of facultative and obligate anaerobes in unsuccessful endodontic treatment.[2] Geurtsen and Leyhausen[12] proposed that the ideal root canal sealer must have both good antimicrobial activity and low toxic effects on surrounding tissue.

The agar diffusion test used in this study is one of the most frequently used methods for assessing the antimicrobial activity of root canal sealers.[13] However, the size of the inhibition zones does not indicate the absolute antimicrobial effect of a sealer. The zones of inhibition may be affected by the diffusibility of the sealer through the

Table 2 Pairwise comparisons of mean diameter of growth inhibition zones, by root canal sealer[a]

	Main group[b]; overall mean diameter of inhibition zones (mm)[c]				
Sealer	**1**	**2**	**3**	**4**	**5**
AH 26	14.25				
Epiphany		19.08			
Diaket		20.33			
Sealapex			22.58		
Sultan				30.00	
Endomethasone					33.83
p value	> 0.99	0.25	> 0.99	> 0.99	> 0.99

[a]*Tukey's honestly significant difference (HSD) test;* $\alpha = 0.05$
[b]*The 6 sealers were categorized into 5 main groups (numbered 1 through 5) according to their effects, AH 26 being the worst and Endomethazone the best.*
[c]*Average of the mean values for all 3 time periods* (n = *12 for each sealer)*

Table 3 Pairwise comparisons of mean width of growth inhibition zones by duration of incubation[a]

	Main group[b]; overall mean diameter of inhibition zones (mm)[c]	
Incubation period	**1**	**2**
72 h	22.20	
48 h	23.13	
24 h		24.71
p value	0.097	>0.99

[a]*Tukey's honestly significant difference (HSD) test;* $\alpha = 0.05$
[b]*The 3 periods of incubation were categorized into 2 main groups according to their effects, the 24-hour incubation being more effective than other periods of time.*
[c]*Average of the mean values for all 6 sealers* (n = *24 for each time period).*

agar, the interaction of the sealer with media components and the microenvironmental conditions in vivo. The root canal sealers evaluated in this study may therefore show different inhibitory effects against *E. faecalis* in vivo.

Today, numerous root canal sealers are available, based on various formulas. Canal sealers containing zinc oxide-eugenol (ZOE) have a strong antibacterial effect.[14] Kaplan and others[15] have stated that the most effective antimicrobial sealers contain eugenol and formaldehyde. The antibacterial effect of the Endomethasone and Sultan sealers, both based on ZOE, depends on the activity of their chemical components. In this study, the Endomethasone and Sultan sealers had the greatest antimicrobial effect against *E. faecalis*. These results confirm those of Grossman,[5] who found that Endomethasone sealer had greater antibacterial capacity than AH 26 and Sealapex sealers.

Some endodontic sealers consist of polymer materials. For example, AH 26 and Diaket are resin-based sealers. The AH 26 sealer has been reported to release a small amount of formaldehyde during the polymerization process, and it is this agent that gives the resin-based sealer its antimicrobial properties.[16] In this study, the AH 26 sealer had the lowest antimicrobial effect against *E. faecalis*, perhaps because only a small amount of formaldehyde was released over a brief period. Diaket is a polyketone compound containing vinyl polymers which, when mixed with zinc oxide and bismuth phosphate, form an adhesive sealer. This sealer is highly toxic in vitro.[17] In this study, it had an antimicrobial effect similar to that of Sealapex and greater than that of both AH 26 and Epiphany.

Root canal sealers with integrated calcium hydroxide, such as Sealapex, have enhanced antibacterial activity.[18] The antimicrobial effect of this sealer is produced by the release of hydroxyl ions, which increases the pH above 12.5. As the calcium hydroxide sealer sets, the pH declines to approximately 9.14, causing a loss of effectiveness.[7]

Sealapex was less effective against *E. faecalis* than Endomethasone and Sultan, because the release of hydroxyl ions was low.[19] However, Sealapex is more effective than AH 26 and the new root canal sealer, Epiphany.

The developers of the Epiphany sealer (Pentron Clinical Technologies) state that this root canal sealer is a dual-curing, resin sealer that is non-mutagenic, non-cytotoxic, biocompatible and resorbable and that it is less irritating than epoxy resin or ZOE sealers. Epiphany showed less antimicrobial activity than all the other sealers in this study, except AH 26. To the authors' knowledge, this is the first study of the antimicrobial properties of Epiphany root canal sealer. It appears that the minimal antimicrobial effect of this material may result from its hydrophilic resin form.

Conclusions

The data reported here indicate that Epiphany root canal sealer was inferior in terms of its antibacterial activity to 4 of the other 5 sealers tested. The Epiphany sealer had an antimicrobial effect equal to or slightly greater than that of AH 26. The new sealer offered no antibacterial advantage over the other sealers tested.

THE AUTHORS

Acknowledgements: *We would like to thank microbiologist S. Bodrumlu, Refik Saydam Hygiene Center, Ankara, Turkey, for the microbiologic evaluation.*

Dr. Bodrumlu *is an assistant professor in the* ***conservative*** *dentistry and endodontics department,* ***faculty of dentistry,*** *Ondokuz Mayis University, Samsun, Turkey.*

Dr. Semiz *is an assistant professor in the department of statistics, faculty of arts and sciences, Selcuk University, Konya, Turkey.*

Correspondence to: *Professor Emre Bodrumlu, Conservative Dentistry and Endodontics Department, Faculty of Dentistry, Ondokuz Mayis University, 55139 Kurupelit-Samsun/Turkey.*

The authors have no declared financial interests in any company manufacturing the types of products mentioned in this article.

References

1. Reit C, Dahlen G. Decision making analysis of endodontic treatment strategies in teeth with apical periodontitis. *Int Endod J* 1988; 21(5):291–9.

2. Sundqvist G, Figdor D, Persson S, Sjogren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1998; 85(1):86–93.

3. Abdulkader A, Duguid R, Saunders EM. The antimicrobial activity of endodontic sealers to anaerobic bacteria. *Int Endod J* 1996; 29(4):280–3.

4. al-Khatib ZZ, Baum RH, Morse DR, Yesilsoy C, Bhambhani S, Furst ML. The antimicrobial effect of various enododontic sealers. *Oral Surg Oral Med Oral Pathol* 1990; 70(6):784–90.

5. Grossman L. Antimicrobial effect of root canal cements. *J Endod* 1980; 6(6):594–7.

6. Siqueira JF Jr, Rocas IN, Souto R, de Uzeda M, Colombo AP. Actinomyces species, streptococci, and Enterococcus faecalis in primary root canal infections. *J Endod* 2002; 28(3):168–72.

7. Bystrom A, Sundqvist G. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. *Int Endod J* 1985; 18(1):35–40.

8. Mickel AK, Nguyen TH, Chogle S. Antimicrobial activity of endodontic sealers on Enterococcus faecalis. *J Endod* 2003; 29(4):257–8.

9. Lai CC, Huang FM, Yang HW, Chan Y, Huang MS, Chou MY, and other. Antimicrobial activity of four root canal sealers against endodontic pathogens. *Clin Oral Investig* 2001; 5(4):236–9.

10. Fabricius L, Dahlen G, Holm SE, Moller AJ. Influence of combinations of oral bacteria on periapical tissues of monkeys. *Scand J Dent Res* 1982; 90(3):200–6.

11. Haapasalo M, Orstavik D. In vitro infection and disinfection of dentinal tubules. *J Dent Res* 1987; 66(8):1375–9.

12. Geurtsen W, Leyhausen G. Biological aspects of root canal filling materials — histocompatibility, cytotoxicity, and mutagenicity. *Clin Oral Investig* 1997; 1(1):5–11.

13. Chong BS, Owadally ID, Pitt Ford TR, Wilson RF. Antibacterial activity of potential retrograde root filling materials. *Endod Dent Traumatol* 1994; 10(2):66–70.

14. Pupo J, Biral RR, Benatti O, Abe A, Vadrighi L. Antimicrobial effects of endodontic filling cements on microorganisms from root canal. *Oral Surg Oral Med Oral Pathol* 1983; 55(6):622–7.

15. Kaplan AE, Picca M, Gonzalez MI, Macchi RL, Molgatini SL. Antimicrobial effect of six endodontic sealers: an in vitro evaluation. *Endod Dent Traumatol* 1999; 15(1):42–5.

16. Leonardo MR, Bezerra da Silva LA, Filho MT, Santana da Silva R. Release of formaldehyde by 4 endodontic sealers. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1999; 88(2):221–5.

17. Pascon EA, Spangberg LS. In vitro cytotoxicity of root canal filling materials: 1. Gutta-percha. *J Endod* 1990; 16(9):429–33.

18. Sjogren U, Figdor D, Spangberg L, Sundqvist G. The antimicrobial effect of calcium hydroxide as a short-term intracanal dressing. *Int Endod J* 1991; 24(3):119–25.

19. Fuss Z, Weiss EI, Shalhav M. Antibacterial activity of calcium hydroxide-containing endodontic sealers on Enterococcus faecalis in vitro. *Int Endod J* 1997; 30(6):397–402.