

An *In vitro* Evaluation of the Antimicrobial Activity of Nine Root Canal Sealers

Leena Smadi, M Dent Sci, FDS RCS; Azmi Mahafzah, PhD;
Ameen Khraisat, PhD



Abstract

Aim: The purpose of this *in vitro* study was to analyze the antimicrobial activity of root canal sealers by using the agar diffusion test (ADT).

Methods and Materials: Three categories of root canal sealers were included in the study: resin-based sealers (4), zinc oxide-based sealers (3), and calcium hydroxide-based sealers (2). The microbial strains used were: *S. aureus* (2 strains), *C. albicans* (2 strains), and *E. faecalis* (1 strain). Statistical analysis was conducted using a one-way analysis of variance (ANOVA). Tests of differences were analyzed using the Tukey's test with a value of $p < 0.05$ considered statistically significant.

Results: The antimicrobial activity of root canal sealers was ranked in descending order as follows: Sealite Regular, Cortisemol, Dentalis KEZ, AH26, Sealapex, Acroseal/Topseal, and Endorez/AH plus.

Conclusion: Root canal sealers showed different inhibitory effects depending on their types and the bacterial strains tested. Root canal sealers containing formaldehyde and eugenol proved to be effective against the microorganisms studied.

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Clinical Significance: The incorporation of antimicrobial components into root canal sealers may become an essential factor in preventing the re-growth of residual bacteria and control of bacteria re-entry into the root canal system.

Keywords: Antimicrobial activity, endodontic treatment, root canal sealers

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Introduction

Endodontic success or failure is related to the absence or presence of signs and symptoms of apical periodontitis¹ which is primarily caused by a bacterial infection of the root canal system.²

Total elimination of microorganisms and their byproducts is the goal of endodontic treatment. The means for achieving this goal are powerful chemo-mechanical debridement, an intracanal dressing, adequate root canal filling, and coronal restoration. However, residual bacteria and fungi have been found in the dentinal tubules, crevices, canal fins, and the ramifications of the root canal system³ even after careful cleaning and shaping of the root canal system. Furthermore, bacteria can penetrate an obturated root canal if the coronal seal is inadequate.⁴ Facultative microorganisms such as *E. faecalis*, *S. aureus* and even *C. albicans* have been considered to be the most resistant species in the oral cavity and possible cause of failure of root canal treatment.⁵

A root canal sealer with antimicrobial activity might better cope with a persistent residual infection and microorganisms re-entering via the

oral cavity, therefore, increasing the chances of a successful endodontic treatment outcome.

The objective of this study was to analyze *in vitro* the antimicrobial activity of nine commercially available root canal sealers against different microorganisms using the agar diffusion test (ADT).

Methods and Materials

Tested Materials

The root canal sealers used in this study are shown in Table 1.

Nine root canal sealers of three types were used in the study as follows:

- A. Resin-based sealers
 1. EndoRez
 2. Topseal
 3. AH26 silver free
 4. AH Plus
- B. Zinc oxide-based (ZOE-based) sealers
 1. Dentalis KEZ
 2. Sealite Regular
 3. Cortisomol
- C. Calcium hydroxide based sealers
 1. Sealapex
 2. Acroseal

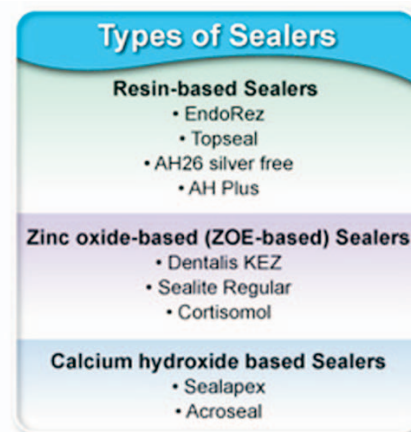


Table 1. Root canal sealers used in this study.

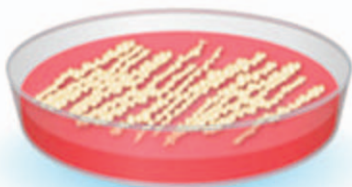
Name	Source	Active ingredients
EndoRez	Ultradent, South Jordan, UT, USA	Urethane dimethacrylate 30%
Topseal	Dentsply, Maillefer, Switzerland	Paste A: Epoxy resin, calcium tungstate, zirconium oxide, aerosol, iron oxide Paste B: Adamantine amine, N,N'-dipenzyl-5-oxanonane-diamine, 1,9-TCD-diamine, calcium tungstate, zirconium oxide, aerosol, silicone oil
AH Plus	Dentsply, DeTrey, Germany	AH plus paste A: Epoxy resins, calcium tungstate, zirconium oxide, silica, iron oxide pigments AH plus paste B: Amines, calcium tungstate, zirconium oxide, silica, silicone oil
AH26 Silver Free	Dentsply, DeTrey, Germany	Powder: Bismuth oxide, Methenamine AH 26 resin: Epoxy resin
Dentalis KEZ	NEO Dental Chemical Products, Tokyo, Japan	Powder: Zinc oxide, calcium hydroxide, iodoform, others. Liquid: Eugenol, others
Sealite Regular	Pierre Rolland, Mengnac, France.	Powder: Diiodothymol, zinc oxide, radio-opacifier, excepients. Liquid: Eugenol, excepients
Cortisomol	Pierre Rolland, Mengnac, France.	Prednisolone acetate 1.1%, paraformaldehyde, zinc oxide, lead oxide red, excipients
Sealapex	Kerr, Romulus, MI, USA.	Calcium oxide 20% Bismuth trioxide 29% Zinc oxide 2.5% Sub-micron silica 3% Titanium dioxide 2% Zinc stearate 1% Tricalcium phosphate 3% Blend 39% Ethyltouene sulfonamide, poly(methylene methyl salicylate)resin, isobutyl salicylate and pigments
Acroseal	Septodent, France Saint-maur, France.	Base: Glycyrrhetic acid (enoxolone), methenamine, radiopaque excipient Catalyst: Calcium hydroxide, DGEBA, radiopaque excipient

All sealers were prepared in strict compliance according to the manufacturers' instructions.

Test Microorganisms

The following microorganisms were used to evaluate the antimicrobial activity of sealers:

- *S. aureus* (ATCC 29213)
- *S. aureus* (ATCC 25923)
- *C. albicans* (NTCC 90028)
- *C. albicans* (NTCC 10231)
- *E. faecalis* (ATCC 29212)



Agar Diffusion Test (ADT)

All sealers were tested using the ADT. *S. aureus* was tested on Mueller-Hinton agar (MH) (Difco, Becton Dickinson France), *E. faecalis* was tested on blood agar (Oxoid, Basingstoke, Hampshire, England), and *C. albicans* was tested on Sabouraud Dextrose agar (Oxoid, Basingstoke, Hampshire, England).

Inocula from a 24 hour growth of the test organisms were added in sterile saline, incubated at 37°C, and allowed to grow to obtain a turbidity equivalent to the 0.5 McFarland standard. A sterile cotton swab was used to evenly streak the test organisms on the agar plates. Wells of 5 mm diameter were punched in agar into which the sealers were added and incubated in a humid atmosphere. Plates were observed after 24 hour, 48 hour, and 7 day intervals at which time the diameter of the zone of inhibition for each sealer was measured and recorded. Sterile saline was used as a negative control. The test was performed in six replicas, and the average reading with a standard deviation was calculated for each sealer tested.

Averages of antimicrobial activity of each root canal sealer on all microorganisms tested and averages of microbial susceptibility of each microbial strain to all root canal sealers used in this study were calculated. It was assumed those values would provide useful summative measures in this study.⁶

Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS) version 12.0 (SPSS, Inc., Chicago, IL, USA) using a one-way analysis of variance (ANOVA). Tests of differences were analyzed using the Tukey's test with a value of $p < 0.05$ being statistically significant at a 95% confidence interval.

Results

Table 2 shows the mean diameter of inhibition haloes (mm) around the agar wells formed by the sealers after exposure to different types of microorganisms. The six replicates were highly reproducible each time the experiment was repeated.

Antimicrobial Activity of Sealers

Endorez sealer showed only a minimal effect against *C. albicans* 90028 and Topseal showed only a minimal effect against *S. aureus* (both 29213 and 25923). Similarly, AH Plus sealer showed only a minimal effect against *S. aureus* 25923. The AH26 sealer was most effective against *S. aureus* 25923 and *E. faecalis* followed by *S. aureus* 29213 and *C. albicans* 90028, but it was not effective against *C. albicans* 10231.

Dentalis was most effective against *C. albicans* 90028 but showed similar antimicrobial activity against *S. aureus* and *C. albicans* 10231 and was not effective against *E. faecalis*. Sealite Regular was most effective against *C. albicans* 90028 followed by *C. albicans* 10231 and *S. aureus* 25923 and showed minimal effect against *S. aureus* 29213 and *E. faecalis*. Cortisomol was most effective against *C. albicans* 90028 followed by *C. albicans* 10231, but it showed similar effect against both types of *S. aureus*, and no effect against *E. faecalis*.

Sealapex was most effective against *C. albicans* 90028. It had a similar effect against *S. aureus* 29213 and *C. albicans* 10231 but was not effective against *S. aureus* 25923 and *E. faecalis*. Acroseal showed minimal effect against both types of *S. aureus* and no effect against *C. albicans* and *E. faecalis*.

The average antimicrobial activity of the sealer for microorganisms in descending order was as follows: Sealite Regular, Cortisemol, Dentalis KEZ, AH26, Sealapex, Acroseal/Topseal, and Endorez/AH plus.

Table 2. Mean diameter of inhibition haloes (mm) formed by sealers after exposure to different types of microorganisms.

Bacteria/Sealer	<i>S. aureus</i> 29213	<i>S. aureus</i> 25923	<i>C. albicans</i> 90028	<i>C. albicans</i> 10231	<i>E. faecalis</i> 29212	Antimicrobial Activity of Sealers on Microorganisms (Mean \pm SD)
Endorez	0	0	10	0	0	2 \pm 4.472
Topseal	10	10	0	0	0	4 \pm 5.477
AH Plus	0	10	0	0	0	2 \pm 4.472
AH 26	10	15	10	0	15	10 \pm 6.124
Dentalis KEZ	10	10	23	10	0	10.6 \pm 8.173
Sealite Regular	10	12	30	19	10	16.2 \pm 8.556
Cortisomol	10	10	22	14	0	11.2 \pm 7.950
Sealapex	14	0	15	10	0	7.8 \pm 7.362
Acroseal	10	10	0	0	0	4 \pm 5.477
Average microbial susceptibility to sealers \pm SD	8.22 \pm 4.842	8.56 \pm 5.126	12.22 \pm 11.144	5.89 \pm 7.457	2.78 \pm 5.652	7.53 \pm 7.585
SD= Standard deviation						

Tukey HSD test showed a significant difference between Endorez and Sealite Regular ($P=0.04$) and between AH plus and Sealite Regular ($P=0.04$).

Microbial Susceptibility to Sealers

S. aureus (29213) was resistant to Endorez and AH plus, while it was slightly susceptible to the rest of the sealers tested. *S. aureus* (25923) was most susceptible to AH 26 followed by Sealite Regular, it was resistant to Endorez and Sealapex, and slightly susceptible to the rest of the sealers tested.

C. albicans 90028 was the most susceptible to Sealite Regular followed by Dentalis, Cortisomol, and Sealapex. It was slightly susceptible to Endorez and AH 26. *C. albicans* 10231 was also the most susceptible to Sealite Regular followed

by Cortisomol, Dentalis, and Sealapex, whereas it was resistant to the rest of the root canal sealers tested.

E. faecalis was most susceptible to AH 26 followed by Sealite Regular and was resistant to the rest of the root canal sealers tested. The average microbial susceptibility to sealers in descending order was as follows: *C. albicans* 90028, *S. aureus* 25923, *S. aureus* 29213, *C. albicans* 10231, and *E. faecalis*.

The ANOVA showed no significant difference in average antimicrobial susceptibility to sealers among the microorganisms tested ($P=0.099$).

Discussion

The ADT has been widely used to test the antimicrobial activity of dental materials.⁶ The

advantage of this method is the creation of direct comparisons of root canal sealers against test microorganisms, and the visual indication of which sealer has the potential to eliminate microorganisms in the local microenvironment of the root canal system.⁷ A disadvantage of the ADT is the results of this method depend not only on the antimicrobial activity of the material for the particular microorganism but it is also highly influenced by the diffusibility of the material across the medium.⁸



The present study tested the antimicrobial activity of nine sealers against microorganisms considered to be resistant to endodontic treatment. This means if a sealer is effective against these resistant microorganisms then it will probably be effective against less resistant microorganisms. In agreement with a previous study⁷ *C. albicans* 90028 was the most susceptible microorganism, whereas *E. faecalis* was the most resistant.

In the present study AH 26, a resin-based sealer, showed antibacterial activity against all tested microorganisms except *C. albicans* 10231. According to Fuss et al.⁹ AH 26 showed good antibacterial activity against *E. faecalis* among the various consistencies of this material.

A previous study¹⁰ investigating the antimicrobial activity of root canal sealers using the ADT determined AH Plus had no antibacterial activity against *E. faecalis*. Another study¹¹ investigated the effects of root canal sealers within dentinal tubules on *E. faecalis* using spectrophotometry and found AH 26 resin-based sealers to have the strongest effect. The antibacterial effect of AH 26

may be related to bisphenol diglycidyl ether which was previously identified as a mutagenic component of resin-based materials.¹² AH 26 also contains hexamethylenetetramine that decomposes into ammonia and formaldehyde in an acid environment.¹³

Cohen et al.¹⁴ measured the amount of formaldehyde released from AH Plus, EZ-Fill, and AH 26 using high performance liquid Chromatography. They found AH 26 yielded the greatest formaldehyde release and the two-paste AH-Plus system had the least amount of formaldehyde release. They determined the relative minute amounts of formaldehyde released by AH-Plus and EZ-Fill did not prohibit their use as root canal sealers. Resin-based sealers Endorez, Topseal, and AH Plus did not show any zone of inhibition. This could be due to the lack of release of formaldehyde.

Antibacterial activity of calcium hydroxide-based materials depends on ionization that releases hydroxyl ions causing an increase in the pH. A pH > 9 may reversibly or irreversibly inactivate cellular membrane enzymes of microorganisms resulting in a loss of biological activity of the cytoplasmic membrane, or leading to the destruction of phospholipids or non-saturated fatty acids that result in a loss of cytoplasmic membrane integrity.¹⁵ Using the ADT, the inefficiency of some calcium hydroxide based sealers might be related to low solubility and diffusibility of these substances in agar.

Kayaoglu et al.¹⁶ investigated the antimicrobial activity of root canal sealers on *E. faecalis* using the membrane-restricted contact test and concluded calcium hydroxide-based sealers, Sealapex, and Apexit were ineffective in reducing the number of cultivable cells of *E. faecalis*. Eldeniz et al.¹⁷ evaluated the antibacterial activity of the resin based sealer, Endorez, in comparison with five other sealers, which included AH 26 and Apexit, using the ADT and Direct Contact Test (DCT). The ADT results indicated Endorez and Apexit showed no antibacterial activity, whereas, the DCT indicated AH 26 was a potent bacterial growth inhibitor.

On the other hand, Mickel et al.¹⁰ showed that antimicrobial activity of Sealapex was second to Roth 811 against *E. faecalis* using the ADT.

Similarly, Sipert et al.¹⁸ used a similar test to the one used in the present study. Using *E. faecalis* 29212, *S. aureus* 25923, and *C. albicans* 10231, Sealapex demonstrated antimicrobial activity for all strains while the resin-based sealer Endorez did not.

The results of the present study using ZOE-based sealers including Dentalis, Sealite Regular, and Cortisomol showed antibacterial activity against all test microorganisms except for *E. faecalis*. Sealite Regular was the only sealer of this type demonstrating antibacterial activity against *E. faecalis*. This confirms previous findings ZOE-based sealers possess a strong antibacterial effect.^{6,10} Eugenol contributes a major part of the antimicrobial activity of those sealers.¹²

None of the sealers tested totally inhibited microbial growth. Thus, endodontic treatment must be performed under aseptic conditions, using powerful chemo-mechanical debridement, an intracanal dressing, adequate filling, and coronal restoration. When a tooth does not respond to root canal treatment, bacteriological

sampling may be needed to determine the bacteria present in the root canal system to facilitate the choice of root canal sealer. The rationale for performing this *in vitro* study is to offer clinicians information regarding the quality and properties of these materials.

Conclusion

Root canal sealers showed different inhibitory effects depending on their types and bacterial strains tested. Root canal sealers containing formaldehyde and eugenol proved to be most effective against the studied microorganisms.

Clinical Significance

The incorporation of antimicrobial components into root canal sealers may become an essential factor in preventing the re-growth of residual bacteria and control of bacteria re-entry into the root canal system. Because the antimicrobial components do not have selective toxicity against microorganisms, they also exert toxic effects on host cells. Therefore, root canal sealers should be used which are characterized by an acceptable biocompatibility.

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About the Authors

Leena Smadi, M Dent Sci, FDS RCS



Dr. Smadi is an Assistant Professor in the Department of Conservative Dentistry and Prosthodontics of the Faculty of Dentistry at the University of Jordan in Amman, Jordan.

e-mail: l.smadi@ju.edu.jo

Azmi Mahafzah, PhD



Dr. Mahafzah is an Associate Professor in the Department of Pathology, Microbiology and Forensic Medicine of the Faculty of Medicine at the University of Jordan in Amman, Jordan.

Ameen Khraisat, PhD



Ameen Khraisat is an Assistant Professor in the Department of Conservative Dentistry and Prosthodontics of the Faculty of Dentistry at the University of Jordan in Amman, Jordan.