



An *in vitro* Comparison of Antimicrobial Activity of Three Endodontic Sealers with Different Composition

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ABSTRACT

Aims: The aim of the study was to compare the antimicrobial property of newly introduced EndoSequence BC sealer with commonly used zinc oxide-eugenol-based sealer (Zical) and epoxy resin-based sealer (MM-Seal) against *Candida albicans*, *Lactobacillus*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

Materials and methods: The agar diffusion test was done to measure the antimicrobial activity of sealers. The sealers were put in the 4 mm wells prepared in the inoculated agar plates. The agar plates were incubated at 37°C for 24 hours and the zones of inhibition that appeared was measured. Chi-square test was done to evaluate intraobserver bias for all study samples. Intergroup comparison was done for all five parameters using Pearson correlation statistical analysis.

Results: EndoSequence BC sealer showed maximum mean of diameter of zones of inhibition against all the microorganisms but with no statistically significant difference with other sealers. Zical did not show any zone of inhibition against the *P. aeruginosa*. MM-Seal did not show any inhibitory activity against the *P. aeruginosa* and *C. albicans*.

Conclusion: EndoSequence BC sealer showed antimicrobial activity against all the microorganisms and proved to be a better choice when compared with other two sealers.

Clinical significance: Antimicrobial properties of endodontic sealers are important to prevent the persistent infection of the complex root canals. EndoSequence BC sealer has been proved to be a better sealer in this aspect.

Keywords: *Candida albicans*, *Endosequence BC*, *Escherichia coli*, *Lactobacillus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*.

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INTRODUCTION

The main objective of root canal treatment is proper cleaning and shaping to reduce the microbial load and helps in healing of the periapex. However, it is difficult to eliminate all the microorganisms from the root canals with biomechanical preparation because of the complex anatomy of the pulp cavity. The endodontic sealers with antimicrobial activity can be helpful to eliminate the remaining microbes from the root canals. Various new sealers are constantly being developed in attempts to meet this criterion.

EndoSequence BC sealer (Brasseler USA, Savannah, GA) is such an example that is based on calcium phosphate silicate.¹ The pH of EndoSequence BC sealer is more than 12 which enhances its bactericidal properties.² The composition includes tricalcium silicate, dicalcium silicate, calcium phosphates, colloidal silica, calcium hydroxide, zirconium oxide (radiopacifier), and water-free thickening vehicles to make it in paste form.³ EndoSequence BC sealer has been promoted as being biocompatible and nontoxic. According to its manufacturer, EndoSequence BC sealer uses the moisture that remains within the dentinal tubules after canal irrigation for its setting reaction.

Epoxy-based resin sealer has been widely used for their long-term dimensional stability, good adhesion to dentin, fluidity, and biocompatibility.^{4,5} MM-Seal

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Table 1: Antimicrobial activity of the sealers against the microorganisms (mean of diameters of zones of inhibition) in mm±SD

Subjects	<i>Candida albicans</i>	<i>Lactobacillus</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
EndoSequence BC sealer	13.43±0.524	23.90±0.739	25.43±0.545	16.33±0.753	14.90±0.629
MM-Seal	0	14.79±0.774	11.07±0.733	10.00±0.588	0
Zical	11.97±0.744	12.40±0.516	13.53±0.548	10.90±0.447	0

(MICRO-MEGA, France) is an epoxy resin-based sealer that has been claimed by the manufacturer to have excellent biocompatibility.

The zinc oxide-eugenol (ZOE) pastes have been commonly used as root canal sealer and are shown to have antimicrobial properties.⁶ Zical (Prevest DenPro, Jammu, India) is a ZOE-based sealer that is based on the Grossman's formula. According to the manufacturer, it has good antibacterial and antiinflammatory properties.

Endodontic infections are polymicrobial which are responsible for the primary infection of the root canals or persistent infection after the root canal treatment. There is very limited data available regarding the antimicrobial activity against the microorganisms used in the study with the three endodontic sealers being tested. The aim of this study was to compare the antimicrobial efficacy of EndoSequence BC sealer, MM-Seal, and Zical against the *Candida albicans*, *Lactobacillus*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* by means of agar diffusion culture test.

MATERIALS AND METHODS

In our study, three root canal sealers, EndoSequence BC sealer (Brasseler USA, Savannah, GA), MM-Seal (MICRO-MEGA, France), and Zical (Prevest DenPro, Jammu, India), were tested for antimicrobial activity against *C. albicans*, *Lactobacillus*, *S. aureus*, *E. coli*, and *P. aeruginosa*. The broth culture suspensions were prepared and adjusted to no. 0.5 McFarland standard. The microorganisms in suspension were spread on 50×100 mm (10 for each microorganism) diameter Petri dishes containing Mueller-Hinton agar medium. The inoculated plates were dried for 15 minutes at 37°C. Three 4 mm well were prepared in each dish with a sterile paper straw from the agar at equidistant. The three endodontic sealers were placed immediately in the wells. MM-Seal and Zical were mixed according to the manufacturer's instruction whereas the EndoSequence BC sealer is a premixed paste. The dishes were kept at room temperature for 2 hours for prediffusion of the material and then incubated at 37°C for 24 hours. After 24 hours the zones of inhibition were observed and measured using millimeter ruler by a single investigator. The data calculated were analyzed statistically.

Chi-square test was done to evaluate intraobserver bias for all five study samples. Intergroup comparison

was done for all five parameters using Pearson correlation statistical analysis. All statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS) version 20 software, and all the results were evaluated at 5% significance level (p-value > 0.05 is insignificant).

RESULTS

The mean diameters of zones of inhibition caused by the three root canal sealers on the microorganisms are presented in Table 1.

In our study EndoSequence BC sealer showed largest mean diameter of zones of inhibition against all the microorganisms followed in sequence of maximum against *S. aureus* (25.5 mm), then *Lactobacilli* (24 mm), *E. coli* (16mm), *P. aeruginosa* (15mm), and *C. albicans* (13.5mm). Similarly MM-Seal showed maximum inhibition against *Lactobacillus* (15 mm), then *S. aureus* (11 mm), and *E. coli* (10 mm). MM-Seal did not show any inhibition against the *P. aeruginosa* and *C. albicans*. Zical showed maximum zone of inhibition against *S. aureus* (14 mm) followed by *Lactobacillus* (12.5 mm), *C. albicans* (12 mm), and *E. coli* (11 mm). This ZOE-based sealer also did not show any inhibitory activity against the *P. aeruginosa*.

EndoSequence BC sealer showed most favorable results as the maximum mean zone of inhibition appeared against all the microorganisms. But statistically no significant difference (p > 0.05) was found between the three sealers. In case of MM-Seal and Zical sealers, where these sealers showed zero inhibitory activity, the statistical analysis could not be performed.

DISCUSSION

The microorganisms used in this study are based on the previous literature, which demonstrates their presence in the persistent or refractory infections of the root canals.^{7,8} *Candida albicans* is one of the most frequent commensal that may become pathogenic yeast in the oral cavity.⁹ *Candida albicans* has been reported to be present in the 21% of the infected root canals.¹⁰ It is found to be causative factor of an acute apical abscess.¹¹ These reports support that *Candida albicans* is involved in the etiology of persistent periradicular lesions. *Candida albicans* is associated with persistent secondary endodontic infections that do not respond to conventional root canal therapy.^{12,13}

Ando and Hoshino¹⁴ studied the composition of the microflora invading the deep layers of human root canal dentin (0.5–2.0 mm from the surface of the root canal wall) and showed that out of 256 predominant bacteria that were isolated, 30% was *Lactobacillus*. Latter is also one of the most frequent species found in the retreatment cases.^{15,16} *Pseudomonas aeruginosa* has been recovered from primary and persistent endodontic infections.^{17,18} Fujii et al¹⁹ recovered *P. aeruginosa* in 6.8% of the bacterial isolates from 20 teeth with persistent apical infections. Leonardo et al²⁰ reported that growth of *P. aeruginosa* was not inhibited by several of the commonly used root canal sealers and pastes.

Escherichia coli was also isolated from persistent periapical infections.¹⁶ Hegde and Lakshmi²¹ found *Escherichia coli* in 15% of the teeth with irreversible pulpitis.

Staphylococcus aureus are Gram-positive facultative anaerobes which are able to remain viable for extended periods because of resistant to drying and temperature changes. Hegde and Lakshmi²¹ reported the presence of *S. aureus* in 2 out of 40 teeth with irreversible pulpitis. Reader CM et al²² reported a case of refractory endodontic lesion due to *S. aureus* and discussed the possible pathogenicity of this microorganism. The agar diffusion method used in our study is one of the most commonly used techniques for evaluating the antimicrobial properties of the dental materials.²³ However, limitations also exist with this method because it does not differentiate between bacteriostatic and bacteriocidal properties of the dental materials and it also do not provide any information about viability of the microorganisms after the test.²⁴

In our study, EndoSequence BC sealer (Brasseler USA, Savannah, GA) showed largest mean of zones of inhibition against all the microorganisms. This may be attributed to its high pH (12.5), hydrophilic nature, and its active calcium hydroxide diffusion.^{2,25,26} Fahd et al²⁷ showed that endosequence root repair material showed excellent antifungal properties. Studies^{28,29} have proven that this material shows similar biocompatibility as mineral trioxide aggregate (MTA) with very less cytotoxicity. Lovato and Sedgley²⁵ showed that the putty and syringable paste had similar antibacterial efficacy when compared to white MTA against 10 strains of *E. faecalis*.

Although the mean zone of inhibition was largest in the EndoSequence BC sealer, but statistically no significant difference was found between the three sealers used in the study. The results were in accordance with Stevens and Grossman,³⁰ Cox et al,³¹ and Orstavik³² who all showed similar inhibitory activity of the ZOE sealers. Hume³³ reported that eugenol present in ZOE sealer is a potent antimicrobial. On the contrary, Zical did not show any inhibitory effects against the *P. aeruginosa*. This is in accordance with the study reported by Leonardo et al.²⁰

Epoxy resin-based sealers have antimicrobial effects related to either bisphenol-diglycidyl ether or the release of formaldehyde during polymerization.³⁴ MM-Seal (MICRO-MEGA, France) showed minimum mean of zone of inhibition against all the microorganisms, except against the *Lactobacillus* which was slightly higher than the Zical. In our study, epoxy resin-based sealer did not show any zone of inhibition against the *Pseudomonas* and *C. albicans*. The low antimicrobial effect of resin-based sealer might be ascribed to the minimal amount of formaldehyde released over time.³⁵ The results are in accordance with the Leonardo et al²⁰ who showed in their study that epoxy resin-based sealer did not show any inhibitory activity against the *Pseudomonas*.

CONCLUSION

Our study showed that EndoSequence BC sealer had profound antimicrobial effect on several types of microorganisms responsible for primary and persistent infections of the root canal. This sealer may suppress the opportunistic microbial growth in the root canals, but it needs to be further related to the *in vivo* studies.

CLINICAL SIGNIFICANCE

The endodontic sealers are the important component of the obturation techniques of the root canals. An endodontic sealer with good antimicrobial properties would help in the long-term success of the primary and retreatment cases of the endodontics.

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