

# *In vitro* comparison of antibacterial properties of bioceramic-based sealer, resin-based sealer and zinc oxide eugenol based sealer and two mineral trioxide aggregates

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## ABSTRACT

**Objective:** Our goal of the study was to evaluate the antibacterial properties of endodontic sealers against the *E. faecalis*. **Materials and Methods:** Six millimeters wells were made for each material in all the preinoculated petri plates. Then, the petri plates were incubated for 24 h. The zones of inhibition appeared were measured, and the measurements were put to statistical analysis. **Results:** EndoSequence BC Sealer, MM-mineral trioxide aggregate (MTA), and ProRoot MTA showed maximum means of diameter of zones of inhibition, whereas MM-seal and Endoseal did not show any zones of inhibition. **Conclusion:** EndoSequence BC Sealer was found to be a better endodontic sealer as compared to resin-based and zinc oxide-eugenol-based sealer.

**Key words:** Antibacterial, endodontic sealer, EndoSequence BC Sealer, *Enterococcus faecalis*, mineral trioxide aggregate

## INTRODUCTION

The successful endodontic treatment in infected teeth depends on the elimination of the microbial load by the chemomechanical preparation of the root canals. However, the complete elimination of microorganism from the root canal system is not possible in all the cases. Endodontic infections are polymicrobial, and more than 150 species of bacteria and other microorganisms are present that are responsible for the primary or persistent infection.<sup>[1]</sup> *Enterococcus faecalis* is a commonly isolated species that may play a

role in persistent endodontic infections. Studies report a prevalence of *E. faecalis* up to 77% in teeth with failed endodontic treatment.<sup>[2,3]</sup>

The use of root canal filling materials having antimicrobial activity is considered advantageous in the effort to reduce the number of remaining microorganisms, prevent recurrent root canal infection, and aid in the healing of periapical tissues.<sup>[4]</sup>

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EndoSequence BC Sealer (Brasseler, Savannah, GA, USA) is a premixed bioceramic endodontic sealer that is mainly composed of zirconium oxide, calcium silicates, calcium phosphate monobasic, calcium hydroxide, filler, and thickening agents.<sup>[5]</sup> Bioceramic sealer has now become popular in endodontics as root repair material and root canal sealer due to its several advantages such as biocompatibility, high pH (<12), nonshrinkable, nonresorbable, ease of delivery in the root canal, and increase in strength of the root the following obturation.<sup>[6,7]</sup>

Endoseal (Prevest Denpro, Jammu, India) is a zinc oxide-eugenol-based permanent root canal sealer with dexamethasone, thymol iodide, hydrocortisone acetate, bismuth subcarbonate, epoxy resins, barium sulfate, and magnesium stearate. According to the manufacturer, it is antibacterial, anti-inflammatory sealant with extended working time, and long setting time.

MM-SEAL (Micro Mega, France) is an epoxy resin-based, paste/paste sealer for permanent filling of root canals using Gutta-percha. According to the manufacturer, it is eugenol-free, biocompatible and radio-opaque.

Mineral trioxide aggregate (MTA) has been proved to exhibit excellent biocompatibility and capacity for inducing mineralized tissue formation and has been used for sealing perforations, for root-end filling, and in apexification cases.<sup>[8]</sup> MTA has demonstrated favorable clinical outcomes in early researches; therefore, other uses for MTA were explored and investigated. MTA has been used as root canal obturation material<sup>[9]</sup> and as root canal sealer after modifications in the original formulation to improve its characteristics.<sup>[10]</sup> It was demonstrated that MTA was very useful as an obturation material in previously treated teeth that are compromised by microleakage, inadequate cleaning and shaping, poor quality obturations, and large periapical lesions.<sup>[9]</sup>

Many studies have been performed to assess the antibacterial activity of different endodontic sealers by different methods. There is little information available about the comparison of the antibacterial properties of the materials used in this study. The goal of the study was to compare the antibacterial activity of the newly introduced EndoSequence BC Sealer (Brasseler, Savannah, GA, USA), MM sealer (Micro Mega, France), Endoseal (prevest denpro, Jammu, India), ProRoot white MTA (Dentsply, Ballaigues,

Switzerland), and MM-MTA (Micro Mega, France) against *E. faecalis*.

## MATERIALS AND METHODS

### Materials used

1. EndoSequence BC sealer (Brasseler, Savannah, GA, USA)
2. MM seal (Micro mega, France)
3. Endoseal (Prevest Denpro, Jammu, India)
4. MM-MTA (Micro Mega, France)
5. ProRoot white MTA (Dentsply, Ballaigues, Switzerland)
6. Microorganism used:
  - a. *E. faecalis* (ATCC 29212).

The activity of the materials was evaluated by the method of agar diffusion test. A base layer of 10 ml of Mueller-Hinton agar was poured in 10 mm × 100 mm sterilized petri plates. The second layer or seed layer containing 10 ml of Mueller-Hinton agar was poured after the solidification of agar and the microbial standardized suspensions were poured. Then, five wells of 6 mm of diameter (one for each material) were obtained by removing the agar at equidistant points with the help of sterilized plastic straw. The wells were immediately filled with all the five materials to be evaluated. The endodontic materials Endoseal, MM seal, and two types of MTA that are MM-MTA and ProRoot MTA were prepared according to the manufacturer's instruction, whereas EndoSequence BC Sealer is a premixed paste.

The plates were incubated at 37°C for 24 h. The inhibition zones around the well were measured with millimeter ruler. The data were collected and subjected to the statistical analysis. Mean (X) and standard deviations were calculated. Chi-square test was done to evaluate intraobserver bias for all the study samples. Intergroup comparison was done using Pearson correlation statistical analysis. All statistical analyses were performed with the SPSS 20.0 statistical software package (IBM).  $P < 0.05$  was considered statistically significant.

## RESULTS

The mean zone of the inhibition recorded from the study has been illustrated in Table 1.

The results of our study showed that the maximum inhibitory activity was shown by the EndoSequence BC Sealer (14.4 mm) followed by MM-MTA (14.3 mm)

**Table 1: Antibacterial activity of the sealers against the *Enterococcus faecalis* (mean of zones of inhibition±standard deviation) in mm**

Endoseal	MM-seal	EndoSequence BC Sealer	ProRoot MTA (Dentsply)	MM-MTA (micro mega)
0	0	14.4±1.497	13.9±1.166	14.3±0.471

MTA: Mineral trioxide aggregate

and the ProRoot MTA the value is 13.9 mm. However, there was no statistically significant difference between these three materials ( $P > 0.05$ ). However, the results showed that no inhibitory activity was found with Endoseal (0 mm) and MM – seal (0 mm) against the *E. faecalis*. The statistical analysis could not be done with these two groups.

## DISCUSSION

*E. faecalis* is a Gram-positive nonspore forming facultative anaerobe bacterium. It has an ability to survive harsh environments. It can resist bile salts, detergents, heavy metals, ethanol, azide & desiccation.<sup>[11]</sup> *E. faecalis* is also resistant against calcium hydroxide, a commonly used intracanal medicament because of its proton pump which maintains the pH level.<sup>[12]</sup> It forms the biofilm which protects it from the host defense and antimicrobial agents.<sup>[13]</sup> It penetrates deep into the dentinal tubules and invades isthmuses, rami, and accessory canals. Many studies have confirmed its presence in failed root canal treatment cases.<sup>[2,14]</sup>

The agar diffusion method was used in our study as it is one of the most commonly used techniques for evaluating the antimicrobial properties of the material. However, this method does not provide an opportunity for the consideration of factors such as anatomy of the tooth and biofilm formation by the microorganism.<sup>[15]</sup>

Our study showed that the maximum mean size of inhibition zone was found with the EndoSequence BC sealer (14.4 mm) followed by MM-MTA (14.3 mm) and ProRoot MTA (13.9 mm). But the results were not significant statistically. This is attributed to high pH released by these materials that promote the elimination of bacteria like *E. faecalis* that do not survive high pH near 11.5 or more.<sup>[16]</sup> Lovato and Sedgley compared antibacterial property of bioceramic root repair material and MTA and their result illustrated similar inhibitory effect against the *E. faecalis*.<sup>[17]</sup> These results are in accordance with our study. Many recent studies have demonstrated similar antibacterial effect against the *E. faecalis* between bioceramic material and MTA.<sup>[18,19]</sup>

MTA was introduced as root perforation material by Lee *et al.* in 1993.<sup>[20]</sup> Further, it gained popularity as pulp capping agent, for pulpotomy, apexification, and as root-end filling material because of its bioactivity and biocompatibility. Now, MTA is being used as the obturation material<sup>[9]</sup> as well as root canal sealer.<sup>[21]</sup> Holland *et al.* demonstrated in their study that MTA used as root canal sealer has potential to regenerate the periodontal ligament and form cementum in the canal system which closes the empty spaces thus, prevent the treatment failure.<sup>[22]</sup> Yildirim and Gencoglu showed successful use of MTA as apical filling material in cases of large apical lesion after 6 years of follow-up.<sup>[23]</sup> Duarte *et al.* demonstrated the mechanism of increase in pH and formation of calcium and hydroxide ions in MTA, which is responsible for the antibacterial effect of MTA. They explained that calcium oxide when reacts with water forms calcium hydroxide which induces high pH that results its dissociation into calcium and hydroxide ions.<sup>[24]</sup>

Epoxy resin-based sealers have antimicrobial effects related to either bisphenol A diglycidyl ether or the release of formaldehyde during polymerization. In our study, MM-seal (Micro mega) did not show any inhibitory effects against *E. faecalis*. Leonardo *et al.* studied the amount of release of the formaldehyde of the four different endodontic sealers and found that epoxy resin-based sealer showed minimum amount of release of formaldehyde during setting.<sup>[25]</sup> This may be the reason that epoxy resin-based sealer used in this study showed no antibacterial activity against the *E. faecalis*. Pizzo *et al.* reported that epoxy resin-based sealer had no inhibitory effect against *E. faecalis* after 24 h,<sup>[26]</sup> which is consistent with our current findings. Slutzky-Goldberg *et al.* tested four endodontic sealers against *E. faecalis* and found that epoxy resin-based sealer AH plus showed no antibacterial activity.<sup>[27]</sup> Zhang *et al.* compared the antibacterial effectiveness of the seven different endodontic sealers using the direct contact method at different time intervals and their results showed that after 24 h, bioceramic-based sealer showed statistically significant high antibacterial activity, whereas epoxy resins-based sealer failed to show any inhibitory effect on the *E. faecalis*.<sup>[4]</sup> Ustun *et al.* evaluated the antibacterial effectiveness of four different sealers AH Plus, EndoRez, MTA Fillapex, iRoot SP against *E. faecalis* by time-kill assay method *in vitro*. They showed that at 20 min, the MTA-based sealer was ineffective, whereas bioceramic sealer and epoxy resin sealer were bactericidal. However, after 7 days and 30 days, MTA-based sealer was bactericidal but all other sealers bacteriostatic.<sup>[28]</sup>



Endoseal (Prevest Denpro), a zinc oxide-eugenol-based sealer did not show any inhibitory effect on the *E. faecalis* in our study. The size of the zone of inhibition depends on two main factors that are toxicity of the material to a particular strain of the bacteria and the ability of the material to diffuse through the particular medium. The diffusibility in turn is affected by three main factors that are hydrophobicity or hydrophilicity of the material, size, and rate of release from the matrix in which the material is put. These factors are difficult to control when the test is being done *in vitro*. Considering these factors, this test may show different results with different sealers.<sup>[29]</sup> Wang *et al.* evaluated the antibacterial effect of four endodontic root canal sealers on *E. faecalis* biofilm in dentinal tubules and found that zinc oxide-eugenol sealer has weaker antibacterial effect as compared to other sealers. They explained the reason on the basis of sensitivity of the technique used to test materials.<sup>[15]</sup> Similarly, Tabrizizadeh and Mohammadi demonstrated low antibacterial effect of zinc oxide-eugenol-based sealer in their study.<sup>[30]</sup> It should be noted that the size of the inhibition zones does not show the exact antimicrobial effect of a sealer. The root canal sealers evaluated in this study; therefore, may show different inhibitory effects against *E. faecalis* *in vivo*.

## CONCLUSION

The bioceramic root canal sealer has similar antibacterial activity as that of both MTAs but better than other two most common types of sealers used. Further research is required to compare their efficacy *in vivo*.

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## Conflicts of interest

There are no conflicts of interest.

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