Original Research

# Comparative evaluation of antimicrobial and antifungal efficacy of bioactive root-end filling materials: An *in vitro* study

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## Abstract

**Context:** Microorganisms are the main cause of failure of endodontic treatment. When retreatment fails periapical surgery followed by retrograde filling is done to seal the apex. A root-end filling material should have antimicrobial property as well as bioactive properties necessary for healing, repair, and regeneration of the apex.

**Aims:** The aim of this study was to evaluate the antibacterial and antifungal efficacy of three bioactive root-end filling materials: mineral trioxide aggregate (MTA) Plus, Biodentine, Endosequence root repair material (ERRM) against *Enterococcus faecalis* and *Candida albicans*.

**Subjects and Methods:** *E. faecalis* and *C. albicans* standard bacterial strains were used. 100 µl was taken from liquid cultures of *E. faecalis* and planted in Mueller-Hinton agar and the same amount of *C. albicans* was planted in Sabouraud dextrose agar by lawn culture. MTA Plus, Biodentine, and ERRM were aseptically filled into the opened pits. Following this, the media were kept in the drying oven at 37°C for 24, 48, and 72 h and the diameters of the inhibition zones were measured.

**Statistical Analysis Used:** Statistical analysis was carried out by Kruskal–Wallis, *Post hoc* (Mann–Whitney), Friedman, and *Post hoc* (Wilcoxon-sign) test.

**Results:** Among the three groups, the antimicrobial activity of Biodentine against *E. faecalis* was statistically higher than MTA Plus and ERRM ($P < 0.05$). Antifungal activity of MTA Plus against *C. albicans* was statistically higher than Biodentine and ERRM ($P < 0.05$). ERRM showed the smallest inhibition zone against *E. faecalis* and *C. albicans* among the three groups ($P < 0.05$).

**Conclusions:** Biodentine exhibited the greatest antimicrobial activity and MTA Plus exhibited the greatest antifungal activity among the three groups. ERRM exhibited the least antibacterial and antifungal activity among the three groups.

**Keywords:** Biodentine; *Candida albicans*; *Enterococcus faecalis*; mineral trioxide aggregate plus

## INTRODUCTION

The main objective of an endodontic procedure is to obtain a 3D hermetic seal between the root canal system and the periodontium. This seal should be present both apically and coronally to prevent leakage as well as to prevent the ingress of oral fluids and microorganisms which can result in reinfection of the tooth. This can result in periapical pathology for which if retreatment does not work, surgical intervention is required. Periapical surgery followed by root-end filling not only removes the diseased periapical tissues and root apex, but it also reseals the root canal system. The purpose of a root-end filling is to provide

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hermetic seal at the root apex to prevent bacteria and its byproducts from entering or leaving the canal.[1]

Infections of the root canal consist of a complex microbial flora of rods, cocci, spirochetes, and fungi. One of the persistent microorganism isolated from these pathological sites are *Enterococcus faecalis* with a prevalence of 24%–77%. The survival skill is due to its ability to bind to dentin and invade and live within the dentinal tubules with the aid of serine protease and binding proteins contained in them.[2] It can maintain pH homeostasis, however *E. faecalis* is unable to survive at pH >11.5.[3] The most common fungi which can be isolated from reinfected root canal are *Candida albicans* with a prevalence of 21%. They are dentinophilic organism having an invasive affinity to dentin. Their size also makes it favorable for invasion of the dentinal tubules. *C. albicans* are able to survive in wide range of pH and are resistant to calcium hydroxide.[4]

A root-end filling material should offer antibacterial and antifungal properties as well as bioactive properties necessary for healing, repair, and regeneration of the apex. Amalgam, glass ionomer cement, cavit, zinc oxide eugenol, Super ethoxy benzioc acid were among the few which was used for root-end filling earlier.[5] These materials failed to meet the ideal requirements of root-end filling material until mineral trioxide aggregate (MTA) was introduced in 1993 by Torabinejad and Chivian and associates. It has the superior sealing ability, biocompatibility, regenerative potential, and the ability to set in the presence of moisture. The main drawback of MTA was its long setting time (3–4 h) and poor resistance to washout.[6] MTA Plus (compounded by Prevest Denpro, Jammu, India for Avalon Biomed, Inc USA) has been introduced in the market with similar constituents as of MTA but with finer particle size and larger surface area. It is supplied with a hydrosoluble gel to decrease the washout property. It has a reduced final setting time of 55 min as compared to the long setting time of MTA.[7]

In recent years, Biodentine (Septodont, Saint-Maur-des-Fosses, France) has gained popularity in the market due to its resemblance with MTA and hence, its wide range of applications where MTA is indicated. It is recommended as a dentin substitute and as a repair material due to its bioactivity, biomineralizing property, good sealing ability, biocompatibility, and good compressive strength.[8]

Endosequence root repair material (ERRM) is a novel root repair material introduced by Brasseler USA, Savannah, GA. It is available in the preloaded syringe or as a premixed putty. Calcium silicate, monocalcium phosphate, zirconium oxide, tantalum pentoxide are the main constituents of ERRM. It has an average setting time of 30+ min. It has nanosphere particles and can enter dentinal tubules. ERRM can set in the presence of moisture in the dentinal tubules. Lovato and Sedgley has proved that ERRM has similar antimicrobial property compared to Proroot MTA.[9] Alsalleeh *et al*. in their study has proved that ERRM has comparable antifungal activity with MTA.[10] However, the antimicrobial and antifungal efficacy of ERRM with Biodentine and MTA Plus has not been compared before. The null hypothesis considered is, there is no significant difference in antimicrobial and antifungal efficacy between the ERRM, Biodentine, and MTA Plus against *E. faecalis* and *C. albicans*.

Thus, the objective of the present *in vitro* study was to analyze the efficacy of MTA Plus, Biodentine, and ERRM against *E. faecalis* and *C. albicans*.

## SUBJECTS AND METHODS

For the study, standard strains, namely *E. faecalis* ATCC 29212 and *C. albicans* ATCC 10231 were used to assess the antimicrobial and antifungal activity. Sterile Brain Heart Infusion broth tubes were inoculated with the standard strains and incubated for 24 h at 37°C. Following incubation, density standard inoculums were prepared by adjusting the broth turbidity to 0.5 McFarland standard with McFarland densitometer for *E. faecalis* and to 0.4 McFarland standard for *C. albicans*. Lawn cultures were prepared from 100 µl standard suspension of *E. faecalis* on sterile dry Mueller Hinton agar (MHA) plates and 100 µl standard suspension of *C. albicans* on sterile Sabourads Dextrose agar (SDA) plates. For *E. faecalis* seven MHA plates and for *C. albicans* seven SDA plates were utilized. Three pits of 5 mm diameter having a depth of 2 mm were prepared on each plate with a sterile punch. All three pits were filled with the three root-end filling materials respectively:

- Group 1-MTA Plus (compounded by Prevest Denpro, Jammu, India for Avalon Biomed, Inc USA)
- Group 2-Biodentine (Septodont, Saint-Maur-des-Fosses, France)
- Group 3-ERRM (Brasseler USA, Savannah, GA).

MTA plus was mixed in powder to liquid ratio of 1:1 and was filled in to its respective pits. Biodentine was prepared according to the manufacturer's instructions in a mechanical triturator and was filled into the pits. Endosequence was syringed directly into the pits. Following this, the plates were incubated at 37°C for 24, 48, and 72 h. At the end of each period, the diameter of the inhibition zone around the pits were measured by measuring the scale in millimeters and the data was recorded.

## RESULTS

Results were obtained and analyzed using SPSS version 20 (IBM Corp, Armonk, NY, USA). Nonparametric tests were used for analysis. Kruskal–Wallis was used to analyze the

mean diameter of the inhibition zone among the groups. *Post hoc* (Mann–Whitney) and *post hoc* (Wilcoxon-sign) test were used to compare the mean diameter of the inhibition zone between the groups and the Friedman test was used to analyze the mean diameter of the inhibition zone within the groups at different time intervals. Results showed that all three groups showed significant antimicrobial and antifungal activity ($P < 0.05$). Inhibition zone formed by MTA Plus, Biodentine, and ERRM against *E. faecalis* and *C. albicans* is shown in Figures 1 and 2 respectively. Among the three groups, the antimicrobial activity of Biodentine against *E. faecalis* was statistically higher than MTA Plus and ERRM [Table 1]. Antifungal activity of MTA Plus against *C. albicans* was statistically higher than Biodentine and ERRM. The zone of inhibition against *C. albicans* at 24 h was the highest which declined at 48 and 72 h progressively [Table 2]. There was no significant difference between the zone of inhibition against *E. faecalis* at 24, 48, and 72 h.

## DISCUSSION

In this study, three bioactive root-end filling materials were used: MTA Plus, Biodentine, and a novel material ERRM against E. faecalis and *C. albicans*. Agar diffusion test (ADT) was used to evaluate the antimicrobial and antifungal efficacy of these three groups. It is one of the most commonly used methods for assessing antimicrobial activity. It allows for direct comparison of the effect of the freshly mixed materials on the microorganism which is to be tested. The efficacy of the ADT depends on the adequate contact between the material and agar gel, diffusibility of the material into the gel, type of microorganism, and cellular density.[11] However, this method has certain disadvantages as it is a qualitative test and it measures the inhibition zone and not the minimal inhibition concentration (MIC).[12] In this study, the flowable paste form of ERRM was used because of the ease of dispensing in to the pits directly through the syringe and also the paste form diffuse more readily through the agar medium which is imperative for the success of ADT.

The results of this study showed statistically significant differences amongst the three groups. Biodentine exhibited the greatest antimicrobial activity against *E. faecalis* followed by MTA Plus and ERRM respectively. This can be because the main constituent of Biodentine powder is tricalcium silicate and its liquid mainly consists of calcium chloride which on combining results in hydration reaction and formation of a colloidal gel and calcium hydroxide. The

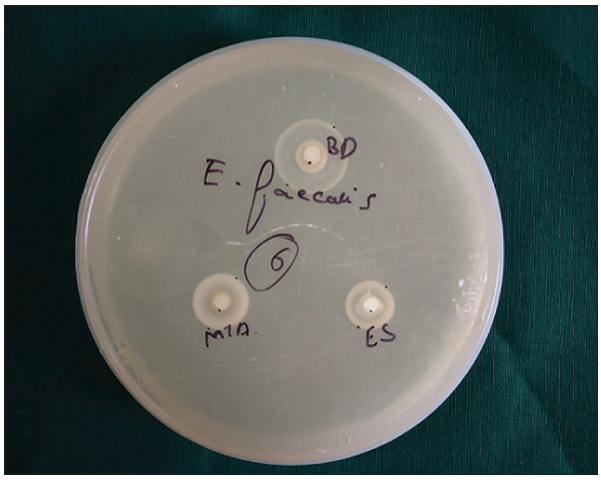


**Figure 1:** Zone of inhibition of *Enterococcus faecalis* at 24 h

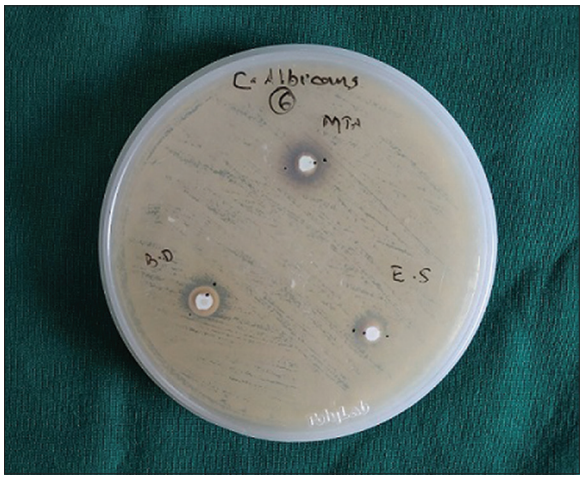


**Figure 2:** Zone of inhibition of *Candida albicans* at 24 h

**Table 1: Comparison of the mean diameter of inhibition zone of *Enterococcus faecalis* among the groups using Kruskal–Wallis**

| Time interval (h) | Groups | Minimum | Maximum | Mean | SD | Median | *P* |
|---|---|---|---|---|---|---|---|
| 24 | MTA plus | 11 | 13 | 12.29 | 0.951 | 13 | 0.001* |
| | Biodentine | 15 | 17 | 16.00 | 0.816 | 16 | |
| | ERRM | 10 | 13 | 11.71 | 1.113 | 12 | |
| 48 | MTA plus | 11 | 14 | 12.29 | 0.951 | 12 | 0.001* |
| | Biodentine | 15 | 18 | 16.00 | 1.155 | 16 | |
| | ERRM | 11 | 13 | 11.57 | 0.976 | 11 | |
| 72 | MTA plus | 11 | 13 | 12.00 | 0.577 | 12 | 0.001* |
| | Biodentine | 15 | 17 | 15.57 | 0.787 | 15 | |
| | ERRM | 11 | 13 | 11.57 | 0.787 | 11 | |

*$P<0.05$. MTA: Mineral trioxide aggregate, ERRM: Endosequence root repair material, SD: Standard deviation

**Table 2: Comparison of the mean diameter of inhibition zone of *Candida albicans* among the groups using Kruskal–Wallis**

| Time interval (h) | Groups | Minimum | Maximum | Mean | SD | Median | *P* |
|---|---|---|---|---|---|---|---|
| 24 | MTA plus | 8 | 11 | 9.57 | 1.134 | 9 | 0.003* |
| | Biodentine | 9 | 10 | 9.14 | 0.378 | 9 | |
| | ERRM | 7 | 9 | 7.57 | 0.787 | 7 | |
| 48 | MTA plus | 7 | 10 | 8.57 | 0.976 | 9 | 0.009* |
| | Biodentine | 7 | 9 | 7.86 | 0.900 | 8 | |
| | ERRM | 6 | 8 | 6.71 | 0.756 | 7 | |
| 72 | MTA plus | 5 | 9 | 7.00 | 1.414 | 7 | 0.027* |
| | Biodentine | 5 | 7 | 5.71 | 0.951 | 5 | |
| | ERRM | 5 | 7 | 5.29 | 0.756 | 5 | |

*$P<0.05$. MTA: Mineral trioxide aggregate, ERRM: Endosequence root repair material, SD: Standard deviation

calcium hydroxide formed subsequently releases $Ca^{2+}$ and $OH^-$ ions.[13] The released hydroxyl ions attack the bacterial cytoplasmic membrane and disrupt cellular metabolism which kills the bacteria.[14] The study by Gandolfi *et al*. proved that compared to Proroot MTA, Biodentine has faster hydration reaction due to higher tricalcium content. There is rapid increase in alkaline pH as well as pronounced calcium and hydroxyl ion release due to their wider pore volume and water sorption that provides wide interative surface for the ion release.[13] The study by Hiremath *et al*. also proved similar results.[15]

According to our study, MTA Plus exhibited the highest antifungal activity compared to Biodentine and ERRM. They contain inorganic powder made of dicalcium and tricalcium silicate along with calcium sulfate, silicon, and bismuth oxide. According to a study by Formosa *et al*., the hydration reaction of MTA Plus is similar to MTA.[7] After mixing, calcium silicates are consumed in the reaction, and calcium hydroxide and calcium silicate hydrate are formed more. The calcium hydroxide formed dissociates to calcium and hydroxyl ions which raises the alkalinity as well as calcium ion release.[16] Torabinejad *et al*. has proved that MTA has a high pH of 10.2 immediately after mixing which increases to 12.5 in 3 h.[17] *E. faecalis* and *C. albicans* cannot withstand this high pH thereby explaining the zone of inhibition around MTA Plus. Another reason could be their fine particle size which means greater surface area for diffusion of $Ca^{2+}$ and $OH^-$ ions.[16] The study by Możyńska *et al*. also reported similar results. They observed higher pH for MTA Plus compared to Biodentine.[18] This could be the reason that MTA plus is a better antifungal agent than Biodentine.

ERRM exhibited statistically significant zone of inhibition around *E. faecalis* and *C. albicans* and had the least diameter of inhibition zone among the 3 groups. The zone of inhibition formed around the pits can be attributed to the basic pH value of ERRM.[10] The reason for its least zone of inhibition may be dependent on the ADT procedure which depends on the diffusibility of the material through the agar.[11] Other types of standard microbial tests also should be done to confirm the same. The literature available on this material is very sparse and further studies need to be done to know the exact setting reaction of the material as well as other properties.

In this study, it was observed that there was no comparable difference between inhibition zone diameter of *E. faecalis* at 48 h and 72 h. This could be due to the initial penetration of materials through the agar gel medium before they set completely at the end of 24 h. This is one of the major drawback of ADT. However, the inhibition zone diameter didn't reduce at 24 h, 48 h, and 72 h proving that antimicrobial effect lasted for 72 h. Whilst for *C. albicans* the zone of inhibition reduced after 24 h proving reduction in antifungal activity. According to Shankar *et al*., yeasts can withstand wide pH variation more than bacteria.[19] This could explain the reason for its resistance compared to *E. faecalis*.

## CONCLUSIONS

Within the limitations of the present study, it can be concluded that Biodentine, MTA Plus, and ERRM exhibited antibacterial and antifungal effects. Among them, Biodentine had the highest antimicrobial activity against *E. faecalis* while MTA Plus had the highest antifungal activity against *C. albicans*. ERRM showed the least antimicrobial and antifungal activity among the three groups.

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### Conflicts of interest
There are no conflicts of interest.

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