# Evaluation of the Antibacterial and Antifungal Activity of Three Retrograde Filling Materials: An In Vitro Study

**Meghna S. Chopra[1], Meenal N. Gulve[2]**


## ABSTRACT

**Introduction:** Retrograde filling materials are used with the purpose of obtaining root end sealing in the endodontic surgeries. In addition to improving the sealing of the existing root canal filling, these materials should possess antimicrobial properties to prevent the movement of bacteria and their products from the root canal system to the periapical tissues for the success of the endodontic surgeries. Study aimed to evaluate the antibacterial and antifungal properties of mineral trioxide aggregate (MTA) compared to biodentine and resin modified glass ionomer cement (RMGIC).

**Material and methods:** Pellets of MTA, Biodentine and RMGIC were prepared to test the influence of these cements on the growth of strains of Enterococcus faecalis, Staphylococcus aureus and Candida albicans using the agar diffusion method. Wells were formed by removing agar and the test materials were manipulated and placed in the wells. The growth inhibition diameter around each material against the tested organism was measured after 24 hour incubation at 37°C. The data was analysed statistically using analysis of variance (ANOVA) and unpaired t-test to compare the differences among the three cements.

**Results:** Inhibition zones for Biodentine against E.faecalis and C.albicans significantly larger ($P<0.01$) when compared to MTA and RMGIC. However RMGIC showed significantly larger ($P<0.01$) zone of inhibition against S.aureus but no zone of inhibition against C.albicans.

**Conclusion:** All materials showed antimicrobial activity against the tested strains except RMGIC against C.albicans. Biodentine had a greater antimicrobial activity compared to MTA and RMGIC.


**Keywords:** Antibacterial activity, Biodentine, Inhibition zone, Mineral Trioxide Aggregate.

## INTRODUCTION

Microorganisms are considered to be the primary etiological agents in the development and progression of pulpal and periapical diseases as well as in endodontic treatment failure.[1] When the healing is not achieved after non-surgical endodontic therapy, and when the retreatment is not possible or failed, surgical approach is required to conserve the tooth. This procedure involves exposure of the involved apex, root resection, preparation of a class I cavity at the resected root end and insertion of a root end filling material in the prepared cavity. The root end filling material should provide an apical seal at the end of the resected root, preventing the movement of the bacteria and the bacterial products from the root canal system to the periapical tissues.[2]

An ideal root end filling material should produce a complete apical seal, be non toxic, biocompatible, non-resorbable, dimensionally stable, easy to manipulate, radio-opaque and well tolerated by the periapical tissues. In addition to these, the root end filling materials should have some antibacterial and antifungal activity to prevent the bacterial and fungal growth.[3]

Glass ionomer cement(GIC) is one of the well known root end filling material which has unique properties such as adhesion to moist tooth structure, low shrinkage and biological acceptance but low antimicrobial activity.[4] In order to overcome the problems of conventional GIC's such as moisture sensitivity, low initial mechanical properties and inferior translucency, a hybrid material combining the technologies of glass ionomer cement and resin composites, Resin modified glass ionomer cement(RMGIC) was developed. RMGIC overcomes the disadvantages of GIC and at the same time maintain their clinical advantages of fluoride release and adhesiveness.[5]

Mineral trioxide aggregate (Angelus,Londrina,PR,Brazil) is marketed as gray and white preparations both of which are composed of 75% Portland cement clinker, 20% Bismuth oxide and 5% gypsum by weight. MTA consists of fine hydrophilic particles that, in the presence of water or moisture forms colloidal gel that solidifies to form hard cement within approximately 4 hours. The white colour preparation lacks tetra calcium aluminoferrite as a result of which, it is more aesthetic.[6]

Biodentine (Septodent) is a calcium based cement recently introduced new endodontic material. The powder mainly consists of tricalcium and dicalcium silicate as well as calcium carbonate. The liquid consists of calcium chloride in aqueous solution with a mixture of polycarboxylate which sets in 12 minutes.[7] Many root end filling materials like amalgam, resin reinforced zinc oxide eugenol cement (Super EBA), Intermediate restorative material (IRM) have been used in the past but due to low sealing ability and antimicrobial activity, there arose a need for the development of these newer retrograde filling materials.

The purpose of this study was to investigate and compare the antibacterial and antifungal effects of MTA, Biodentine and RMGIC on Enterococcus faecalis, Staphylococcus aureus and Candida albicans microorganisms.

## MATERIAL AND METHODS

The study was performed in the Department of Microbiology at the Karmaveer Bhaurao Patil (KBP) College, Vashi, Navi Mumbai, India. The test materials- MTA (Angelus,

[1]PG Student, [2]Professor and Head, Department of Conservative Dentistry and Endodontics, MGV's KBH Dental College and Hospital, Nashik, Maharashtra, India

**Corresponding author:** Dr. Meghna S. Chopra, 705, Natasha Towers, 7 Bungalows, Andheri(west), Mumbai-400061, India.

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Londrina, PR Brazil), Biodentine (Septodent) and RMGIC (Vitremer, 3M ESPE, St.Paul, MN, USA) were manipulated strictly in accordance to the manufacturer's instructions. The antimicrobial activity of the endodontic cements was evaluated by the agar diffusion method against three strains: Enterococcus faecalis (ATCC 29212), Staphylococcus aureus (ATCC 25923) and Candida albicans (ATCC 10231). Bacteria were diluted to obtain a suspension of approximately $5x10^6$ colony forming units/ml in sterile Tripticase Soy Broth (TSB) obtained by spectrophotometer. 10 ml of Mueller Hinton agar was poured in Petri plates to form a base layer. When it solidified, a second layer or seed layer containing 10 ml of Mueller Hinton agar and 200 µl of the microbial standardized suspensions was poured over it. After solidification of the second layer, three wells of 7 mm of diameter (one for each material) were obtained by removing a standardized portion of the agar in equidistant points with the aid of sterilized cork-borer and immediately filled with freshly manipulated test materials. After prediffusion of the test materials for 2 hours at room temperature, all the plates were incubated at 37°C and evaluated at 24 hours.

## STATISTICAL ANALYSIS

Microbial inhibition zones were measured with a 0.5mm precision ruler and results were expressed as mean and standard deviation. The data was analysed statistically by one way analysis of variance (ANOVA) at 95% confidence interval and 2 degrees of freedom and Unpaired t-test using Statistical Package for Social Sciences (SPSS) software version 21 (SPSS Inc, Chicago,IL, USA).

## RESULTS

The antimicrobial activities of MTA, Biodentine and RMGIC and the mean and the standard deviation values are shown are listed in Table-1. The inhibition zones shown by MTA, Biodentine and RMGIC are shown in figure-1, figure-2 and figure-3 respectively. Biodentine showed significantly (greater activity against all the three microorganisms when compared to MTA ($P<0.01$). The difference was significant ($P<0.01$) when compared to RMGIC against E.faecalis and C.albicans. RMGIC significantly ($P<0.01$) inhibited S.aureus when compared to MTA and Biodentine, however it was incapable of inhibiting C.albicans.

## DISCUSSION

The strains evaluated in this study were E.faecalis, S.aureus and C.albicans, which are frequently isolated during routine endodontic treatment of an infected root or from teeth with periapical pathology. They have been found with higher frequency in cases in which treatment had been protracted, in flare-ups and in failing cases. These micro-organisms can enter the root canal system before or after treatment and may then cause secondary infections. E.faecalis was chosen as the test organism because it is associated with persistent apical inflammation in clinical situations. It has been used extensively in endodontic research because it has been found in infected canal and has been associated with failed root canal treatment.[8] S.aureus is predominantly found in persistent or refractory periapical lesions in teeth subjected to periapical surgery.[9] Fungi such as C.albicans may gain access to the root canal from the oral cavity as a result of poor asepsis during endodontic procedures, or because of coronal leakage. C. albicans has the ability to form biofims on different surfaces and may be involved in cases of persistent and secondary infection.[10] The agar diffusion method has been widely used method for evaluation of antimicrobial activity in vitro and reflects intrinsic antibacterial activity and diffusivity of the materials examined.

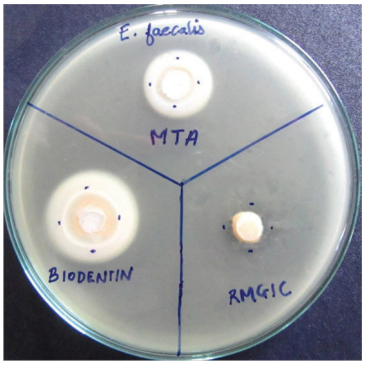


**Figure-1:** Inhibition zones against E.faecalis by the three test materials

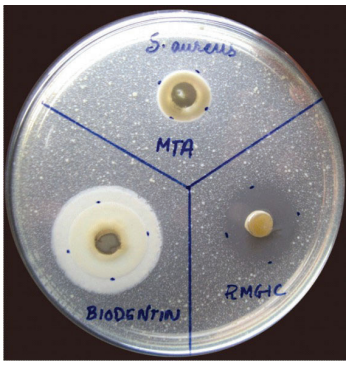


**Figure-2:** Inhibition zones against S.aureus by the three test materials

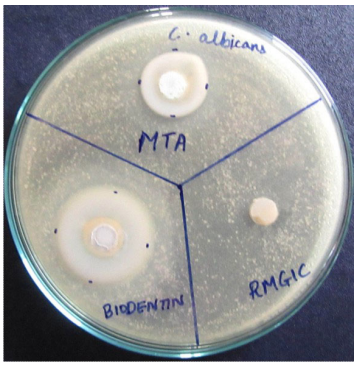


**Figure-3:** Inhibition zones against C.albicans by the three test materials

| Test material | E.faecalis | | S.aureus | | C.albicans | |
|---|---|---|---|---|---|---|
| | Mean | S.D | Mean | S.D | Mean | SD |
| MTA | 13.16 | 0.28 | 14.5 | 0.5 | 18.16 | 0.28 |
| Biodentine | 14.66 | 0.57 | 21.16 | 0.28 | 24.33 | 0.57 |
| RMGIC | 12.33 | 0.28 | 22.5 | 0.5 | 0 | 0 |

**Table-1:** Mean and standard deviation (S.D) of inhibition zones in Millimeters

It allows direct comparison of medicaments against the test microorganisms, indicating which medication has the potential to eliminate bacteria from the root canal system.[11]

In the present study, Biodentine showed higher inhibition halos against E.faecalis and C.albicans as compared to MTA and RMGIC. Biodentine is available in the form of powder and liquid which can be mixed in an amalgamator. The main component of powder is a tricalcium silicate, with the addition to the powder of Calcium carbonate ($CaCO_3$) and Zirconium oxide ($ZrO_2$).The liquid is a solution of Calcium chloride ($CaCl_2$) with a water reducing agent. On setting it forms a gel structure which results in the ionic exchange. Compared to other calcium based cements, it presents a faster setting time of about 12 minutes and higher mechanical properties as the compressive strength is 241 MPa and flexural strength is calculated to 34 MPa. Thus it is mechanically stronger, less soluble, gives a tighter seal preventing microleakages. Antimicrobial efficacy of Biodentine can be assumed to be the result of hydration of tricalcium silicate resulting in formation of colloidal gel and release of calcium hydroxide, which provides the ability to inhibit micro-organisms.[12] Hence in this study Biodentine had a significantly higher antimicrobial activity as compared to MTA.

MTA contains calcium oxide, which when mixed with water, forms calcium hydroxide, inducing an increase of pH by dissociation of calcium and hydroxide ions. Antimicrobial action of MTA is attributed to its high initial pH of 10.2 which rises to 12.5 in 3 hours.[13] MTA showed antifungal activity against C.albicans which was in agreement to the study by Al-Nazhan and Al-Judai.[14]

RMGIC has formed the highest inhibition zone against S.aureus as compared to MTA and Biodentine whereas no inhibition zone was formed against C.albicans. The inclusion of Fluorite and/or cryolite as fluxes for firing purposes in the RMGIC may enhance the release of fluoride ions into the matrix during the setting reaction in the initial 24 hour period. Furthermore, the liquid component of RMGIC conventionally contains hydroxy-ethyl methacrylate, which in part may aid the antibacterial effect by providing a low initial pH. The initial mix of the cement is also of low pH (2.2-3.6) and rises to neutrality during the progress of the setting reaction. This early acidity may play a major role in its antibacterial effect.[15,16] No previous studies have evaluated the antifungal activity of RMGIC. Anna Carolina et al., demonstrated no activity of GIC against C.albicans which could be due to the fluoride concentration released by the material.[17] The results of this study suggest that Candida is resistant to RMGIC as there is no significant zone of inhibition formed around it.

## CONCLUSION

All the materials showed antimicrobial activity against the tested strains except RMGIC on Candida albicans. Biodentine created larger inhibition zones against E.faecalis and C.albicans compared to MTA and RMGIC. Biodentine significantly inhibited all the microorganisms. Thus we conclude that Biodentine has a greater antimicrobial activity as compared to MTA and RMGIC. However variations in agar medium, bacterial strains, diffusion capacity of inhibitory agents and cellular density may interfere with the formation of inhibition zones around materials used in antimicrobial testing.

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