In vitro comparative evaluation of apical microleakage with three different root-end filling materials

Dr. Nalini Desai, Dr. Rajeev S, Dr. Sahana S, Dr. Jayalakshmi KB, Dr. Hemalatha B, Dr. Sivaji K, Dr. Vijay Kumar R, Dr. Vamshi Krishna V, Dr. Savitri D, Dr. Gyanendra Pratap Singh

Abstract
Aim: To compare and evaluate the microleakage of three root end filling materials - Mineral Trioxide Aggregate (MTA), Biodentine and bioaggregate using vacuum dye penetration technique.

Materials and methodology: Thirty extracted single rooted premolars were selected and decoronated. Root canals of teeth were cleaned, shaped using hand protaper file and obturated with corresponding gutta percha and AH-plus sealer. The teeth were stored in saline. After 1 week, apical 3mm of the root was resected at an angle of 90° to the long axis of the root. Teeth were divided into three experimental groups of six teeth each according to the filling material. The root surfaces were covered with nail varnish up to 2mm from the apical foramen, then immersed in 0.2% Rhodamine B solution in a vacuum chamber and maintained in the dye at 37 °C and 100% relative humidity for 24 hours for evaluation of marginal leakage. The teeth were then sectioned longitudinally & examined under stereomicroscope. The depth of dye penetration was measured in millimeters and statistical analysis was carried out. Statistical analysis was carried out using one way ANOVA test.

Results: Comparison of microleakage showed maximum peak value of 0.58 mm with a standard deviation of 0.37mm with a standard deviation of 0.14 for MTA, 0.22 with a standard deviation of 0.13 for biodentine and 0.22 with a standard deviation of 0.13 for bioaggregate. Results showed p < 0.003, which was statistically significant.

Conclusion: Thus it can be concluded that Bioaggregate showed a better result as root end filling material to prevent microleakage, in comparison to biodentine and MTA.

Keywords: Collum, root, angle, cephalogram

1. Introduction
The primary aim of root canal treatment is the elimination and exclusion of all the microorganisms from the root canal system. Despite the constant evolution of concepts, new endodontic techniques and the development of more effective materials and instruments, the resolution of periapical pathosis is not achieved in certain cases. In such cases, where conventional endodontic treatment is unsuccessful, surgical endodontic therapy is needed to save the tooth. This procedure includes exposure of the involved apex, resection of apical end of root, preparation of retro-class I cavity and insertion of a root end filling material [1, 2].

An ideal root-end filling material should adhere to the prepared cavity walls forming a tight seal in the root canal system. It should be easy to manipulate, radiopaque, dimensionally stable, and nonabsorbable, adhesive to dentin, nontoxic, well tolerated by the periradicular tissues and promote healing [3]. Among materials present, the choice is based on handling properties, biocompatibility, apical seal and long term clinical success. Many materials have been introduced for this purpose in endodontic surgery such as gutta-percha, amalgam, Cavit, intermediate restorative material (IRM), Super EBA, glass ionomers, composite resins, carboxylate cements, zinc phosphate cements, zinc-oxide eugenol cements. Recently, Mineral trioxide aggregate (MTA), biodentine, bioaggregate have also been used [2, 3, 4].

When non-adhesive materials are used for apical sealing, a microscopic space always exists between the restoration and the tooth which leads to microleakage. Microleakage is defined as flow of oral fluid and bacteria into the microscopic gap between a prepared tooth surface and a restorative material [2, 5, 6, 7].
The objective of this study was to evaluate the apical leakage of root-end preparations filled with mineral trioxide aggregate, biodentine and bioaggregate.

**Material and Methodology**

**Study Samples**

30 extracted single rooted premolars with completely formed apices and straight canals were taken for this study. The samples were collected from Krishnadevaraya College of dental sciences extracted for orthodontic purposes. The teeth were cleaned using ultrasonics and were decoronated using a diamond disc at CEJ.

![Fig 1: decoronated sample.](image1)

**Sample Processing**

Preoperative radiographs were taken to confirm the single canal anatomy. The pulp tissue was extirpated using barbed broach. K-file was used to confirm the canal patency, working length was determined. A glide path was prepared till the working length with #20 K-File. Canals were then prepared using the complete sequence of hand protaper files using 3% sodium hypochlorite and 17% EDTA as irrigants. Canals were obturated with gutta percha by lateral compaction technique. Radiographs were taken to confirm the quality of obturation and the access cavities were sealed with composite resin restorative material after 24 hours. The teeth were then stored in saline for 1 week. They were resected apically at 90° angle axis to the long axis of the root using crosscut fissure bur (556, Mani, Japan) removing 3 mm of the apex. The 3mm x 1mm deep retrograde cavity was prepared using straight fissure diamond bur SF-41, the cavities were irrigated with saline and dried.

![Fig 2: sample with retrograde cavity preparation done.](image2)

**Vacuum dye penetration method**

The rhodamine B dye was applied in a vacuum environment of 20 mmHg maintained by a vacuum pump. The samples were kept in the vacuum for one hour and then were maintained in the dye at 37 °C and 100% relative humidity for 24 hours. After the time elapsed, the roots were washed in running water and dried. The teeth were then sectioned longitudinally. The dye penetration was examined under stereomicroscope at 30X magnification and microleakage was evaluated in millimeters.

**Stereomicroscopic evaluation**

![Fig 3: dye penetration along the margin of the retrograde filling materials and tooth structure: a) MTA b) Biodentine c) Bioaggregate.](image3)

**Results**

The mean dye penetration values (mm) are shown in Table 1. The results were analyzed by non-parametric one way ANOVA test at a significance level of 5%. (p>0.05).

Table 1 shows microleakage values for different groups. Comparison of microleakage showed maximum peak value of 0.58mm with a standard deviation of 0.14 for MTA, 0.37mm with a standard deviation of 0.16 for Biodentine and 0.22mm with a standard deviation of 0.13for Bioaggregate. Results of one-way ANOVA test for leakage showed p < 0.001, which is statistically significant. Microleakage was found to be significantly less in Bioaggregate (0.22 mm) when compared to Biodentine (0.37 mm) (p < 0.001) and with MTA (0.58 mm) (p < 0.001).

~ 30 ~
The quality of apical seal obtained by root end filling materials has been assessed by the degree of dye penetration, radioisotope penetration, bacterial penetration, electrochemical means and fluid filtration techniques [7, 8]. All of these techniques have been shown to have a variety of shortcomings. The dye penetration method used for measuring sealing ability is the most popular and is easily performed. Leakage is appreciated considerably higher when the vacuum dye penetration method using rhodamine B dye is done [8]. MTA has been investigated and used as a root end filling material since its introduction. Despite its good physical, biological properties and its hydrophilic nature, MTA has some disadvantages such as long setting time and high cost. The search for alternative materials is aimed to reduce costs and to increase the feasibility to both professional and patient [6, 9, 10, 11]. BioDentine is similar to MTA in basic composition. The manufacturers claim that it’s modified powder composition i.e the addition of setting accelerators and softeners, a new predosed capsule formulation for use in a mixing device largely improve the physical properties of the material making it more user-friendly. Biodentine proves superior to MTA as the setting is faster there is a lower risk of bacterial contamination. The results of this study showed that all materials exhibited microleakage but there was significantly less leakage in Biodentine (0.13 mm) when compared to MTA (0.73 mm) [12, 13].

Bioaggregate is a relatively new bioceramic based material which has nanosized particles that achieve excellent adhesion to the dentinal walls of the root canal. It shows excellent biocompatibility and significant stimulation of periodontal ligament. According to the present study, bioaggregate showed the least amount of microleakage; surprisingly even when compared to MTA, though both have nearly the same components. Bioaggregate is composed of tricalcium silicate, tantalum oxide, calcium phosphate and silicon dioxide and is free of aluminium. On hydration, tricalcium silicate produced calcium silicate hydrate and calcium hydroxide. Our results are in agreement with the previous studies which indicated that ceramic based materials ensure much better apical seal than IRM, amalgam or super EBA materials. The hermetic seal associated with this bioaggregate could be because of the nanosized particles that achieve excellent adhesion to the dentinal walls of the root canal and its hydrophilic nature demonstrating setting expansion. The presence of a gel-like calcium silicate hydrate as main structural component in both the MTA and bioaggregate provides strength, hardness and sealing properties to the set material [12, 13]. Vacuum dye penetration method was used in this study as the vacuum allows a more accurate assessment of dye penetration and depth of dye penetration varies according to the amount of air entrapped within the canals. In this study rhodamine B dye was chosen due to its smaller and more surface active molecules. In addition it does not undergo changes in the presence of substances rich in calcium [7, 13, 14]. This study showed that all materials exhibited microleakage but there was significantly less leakage in bioaggregate and Biodentine when compared to MTA.

**Table 1: Mean dye penetration values**

<table>
<thead>
<tr>
<th>Materials</th>
<th>Mta</th>
<th>Biodentine</th>
<th>Bioaggregate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum Leakage</td>
<td>0.4mm</td>
<td>0.2mm</td>
<td>0.1mm</td>
</tr>
<tr>
<td>Maximum Leakage</td>
<td>0.8mm</td>
<td>0.6mm</td>
<td>0.4mm</td>
</tr>
<tr>
<td>Mean+/- Sd</td>
<td>0.58+/-0.14</td>
<td>0.37+/-0.16</td>
<td>0.22+/-0.13</td>
</tr>
</tbody>
</table>

Significance by ANOVA: F=20.449, p<0.001

**Graph:** The range variation among dye leakage values of three materials (mm)

**Discussion**
Most *in vitro* studies evaluate leakage of the apical seals, but the correlation between dye leakage around root-end filling materials and their clinical performance is uncertain. The clinical significance of microleakage in apical surgery has not been elucidated. However it seems logical that the lesser leakage would prevent migration of bacteria and toxins into the periradicular tissue.

**Conclusion**
The results of this study showed that all materials tested were unable to avoid dye penetration. Despite the differences bioaggregate showed least microleakage compared to biodentine and MTA.

**References**

~31~