



RESEARCH ARTICLE

TO EVALUATE AND COMPARE TWO NEWER MATERIALS AS ROOT END MATERIAL FOR BACTERIAL MICROLEAKAGE – AN IN VITRO STUDY

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ABSTRACT

Background: The main cause of pulpal and periradicular pathology are microorganisms and their by-products in the root canal system. Teeth involving periapical pathology requires root canal treatment with hermetic seal. But sometimes periapical healing is not achieved even after conventional root canal treatment. Previously different leakage tests have been done to check the sealing ability of newer root end filling materials but never before bacterial leakage study has been done on newer root end filling materials.

Aim: The aim of this study was to compare the bacterial leakage & CFU count of MTA & Biodentine used as a root-end fillings material.

Results: Both bacterial microleakage and CFU count results showed statistically non-significant results between Group A (MTA) & Group B (Biodentine). But Biodentine had an edge over MTA.

Conclusion: Although the results of the study were non-significant, but Biodentine opens a new avenue as root-end filling material. It certainly holds better possibilities on the horizon over MTA.

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INTRODUCTION

The oral cavity is similar to other sites of the body as it is colonized with normal flora in symbiotic relation. If the normal flora is provided with the right conditions along with gaining access to a normally sterile tissue such as the dental pulp or peri radicular tissue, they become opportunistic pathogens. (Craig Baumgartner, 2004) Pertaining to teeth the root canal anatomy is highly complex. Even after instrumentation, irrigation, and intracanal medication, bacteria might still be found inside the intricate root canal system with the potential for diseases to persist or emerge. (Bystrom and Sundqvist, 1983; Orstavik, 1981) Moreover, a closed root space has low oxygen content. Anaerobic bacteria are thus given an ideal atmosphere to live, grow, and ultimately activate the vast immunological defence systems that result in host destruction. Root canal therapy is the most commonly used method to treat irreversible pulpitis and periradicular inflammation. It involves three steps: root canal preparation, root canal dressing, and root canal obturation. Complete sealing of the entire canal space is

an important factor to the success of root canal treatment. Inadequate obturation, improper apical seal etc. are among various factors which risks of fluid penetration through the obturated root canal to the root apex. Fluids such as bacteria-laden saliva and tissue fluids become irritants after degradation, leading to periradicular inflammation. (Wang et al., 2012) Most endodontic failures occur as a result of leakage of irritants from pathologically involved root canals. When non-surgical attempts prove unsuccessful or are contraindicated, surgical endodontic therapy is needed to save the tooth. The root-end filling material should provide an apical seal to an otherwise unobturated root canal or improve the seal of existing root canal filling material and be biocompatible with the periradicular tissue. (Vasudev and Goel, 2003) Gartner and Dorn (1992) proposed that an ideal root-end filling material should be easy to manipulate, radiopaque, dimensionally stable, non-absorbable, insensitive to moisture, adhesive to dentin, nontoxic, and biocompatible. Although a plethora of materials are available, no material has been found that fulfils all or most of the properties for ideal retrograde filling material. (Vasudev and Goel, 2003) Off late two newer materials have been widely used as root end filling material; MTA & Biodentine. Since there is paucity of information available about the use of these two materials as root – end filling material and its ability to prevent bacterial microleakage, this study was planned.

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Aims

- 1) To assess the bacterial microleakage through MTA & biodentine as root end filling material.
- 2) To compare the bacterial microleakage of MTA & biodentine as root end filling material through CFU count.

MATERIALS AND METHODS

The study sample comprised of 20 caries free teeth which were divided into two groups, each comprising of 10 teeth and labelled as Group A (MTA) and Group B (Biodentine). Before the teeth were used, they were washed in distilled water and later cleaned with aqueous slurry of pumice using handpiece and rubber cup. Initial radiographs were taken to check the patency of the canals and standard access cavities were prepared. In all the 20 samples, coronal portion of each canal was enlarged with #2 to #4 Gates Glidden drills. In order to obtain a standardized diameter, the apical foramina of the teeth was enlarged and kept patent to a #40 file, using a step-back filing technique. Approximately 2 ml of 5.25% NaOCl was used between each file size, to remove debris. Root canals were then obturated to the desired working length and the coronal portion was sealed with a temporary restoration Cavitemp (Ammdent). The apex of the selected teeth were cut and apical cavity of 3 mm was prepared in order to eliminate any lateral canals or apical ramification. Nail varnish was applied to the surfaces of all teeth excluding the resected apical portion in order to prevent bacterial microleakage through lateral canals or other discontinuities in the cementum. The apical cavity in Group A samples were sealed with MTA (ProRoot) (Fig. 1) and in Group B with Biodentine (Septodont) (Fig. 2). The MTA and Biodentine was mixed with sterile water on paper pad for the two groups respectively and filled into the prepared cavity. The samples of Group A were then kept in a moist cotton pellet for 6 hours for setting. Group B samples were kept in moist cotton pellet for 15 min. for setting. A 25-mm length of tubing was placed over the coronal portion of each tooth and the edges were sealed. By using a high-speed handpiece and #2 round bur, a small circular opening (about 1 mm in diameter) was made through the cap of a 30-ml sterilized flask. A paper clip was threaded through the opening and was hung on the inside. The outside end was bent to stabilize the clip against the cap. The outside opening of the cap was resealed. The tubes, with the teeth attached, were sterilized in 5.25% sodium hypochlorite and then were rinsed with approximately with 300 ml of sterile water. The tubes above mentioned were then fastened to the caps of the previously sterilized vials. Once the preparation was done, 10ml of sterile phenol red broth with 3% lactose was added to the bottom of a flask, and the length of the tubing was adjusted so that a minimum of 2 mm of the apical part of each tooth was immersed in the solution. Three different species of bacteria (*P. vulgaris*, *S. epidermidis* and *E. fecalis*) were used as contaminants for this experiment; the coronal portion of the root canals of 20 teeth was placed in contact with 2 ml of bacterial contaminants and 0.7 ml of sterile artificial saliva. The samples were monitored daily until the red indicator solution at the bottom of the flask turned yellow (Fig. 3). This red indicator solution was then analysed using culture media

and colony forming units of bacteria were counted and tabulated (Fig 4 &5). The scores thus obtained were subjected for statistically analysed using Chi-square and Paired t-test.



Fig. 1. Group A (MTA) Samples



Fig. 2. Group B (Biodentine) Samples



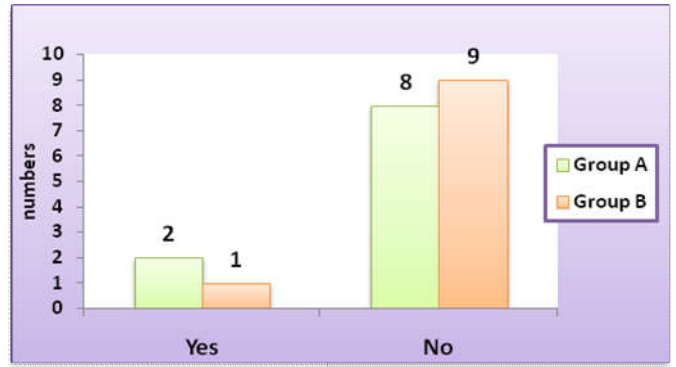
Fig. 3. Visible colour Bacteria Specimen



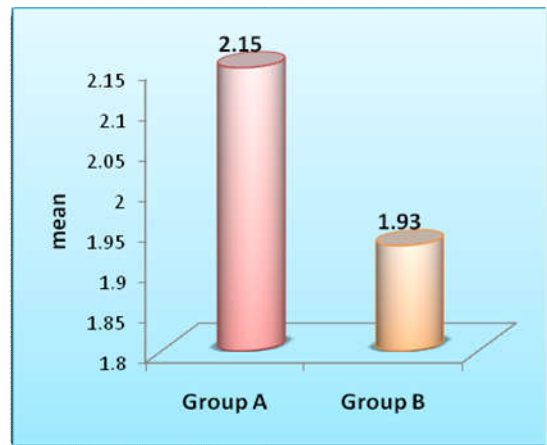
Fig. 4. Culture of bacteria for CFU count in Group A



Fig. 5. Culture of bacteria for CFU count in Group B



Graph 2. Bacterial microleakage at 4week in Group A and Group B



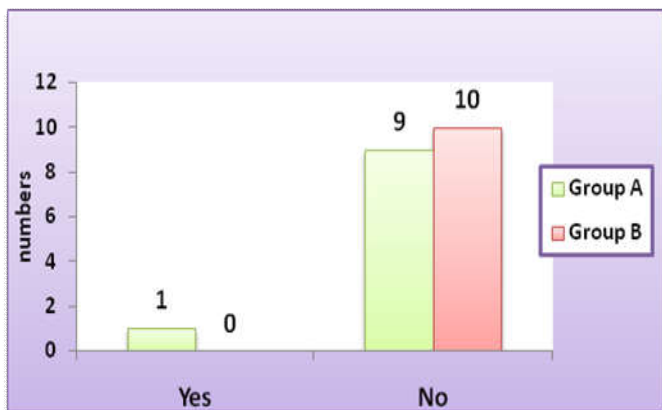
Graph 3. CFU in Group A and Group B

RESULTS

Both groups showed bacterial microleakage – 1 sample of Group A at the end of 3rd week and 1 of Biodentin at the end of 4th week which was statistically not significant (GRAPH 1& 2). Mean CFU in Group A was $2.15 \times 10^5 \pm 0.208 \times 10^5$ and in Group B it was $1.93 \times 10^5 \pm 0.15 \times 10^5$. Mean CFU was less in Group B compare to mean CFU in Group A but there was no statistically significant difference in Bacterial micro leakage in Group A and Group B (Graph 3).

DISCUSSION

The main cause of pulpal and periradicularpathosis are microorganisms and their by-products in the root canal system. (Haapasolo, 1989) The relationship between bacterial infection of dental pulp and periapical lesion formation has been elegantly demonstrated in the classic studies of Kakehashi *et al.* (1965). Convincing evidences have accumulated that infections of root canals are polymicrobial infections where anaerobes predominate. Among various facultative anaerobes which are seen in periapex, S.epidermis is frequently encountered and is seen present in periapical inflammatory lesions as it is believed to be introduced into the root canal and possibly goes beyond the apical foramen during the initial root canal treatment. (Orstavik, 1981; Wang et al., 2012) The bacterial species *E. faecalis* a part of normal flora in humans and is associated with different forms of periradicular disease



Graph 1- Bacterial microleakage at the end of 3week in Group A and Group B

including primary endodontic infections and persistent infection. In the category of primary endodontic infections, *E. faecalis* associated with asymptomatic chronic periradicular lesions significantly more often than with acute periradicular periodontitis or acute infections. (Sundqvist and Figdor, 2003) In post treatment apical periodontitis, the prevalence ranges from 24% - 77%. (Sundqvist and Figdor, 2003) *P. Vulgaris* is a highly motile organism. Hence all these three bacterial species because of the various reasons stated above were selected for the study. Root end resection is an important component of endodontic surgery as it will aid in eliminating anatomical variations, resorptive defects, ledges, perforation defects, canal obstructions, and separated instruments that may be present in this area of the root. (Camilleri, 2008) As apical microleakage is the most common cause responsible for failure of endodontic therapy, it is important to prevent this by sealing the root ends with suitable root end filling material. Hence, selection of ideal root end filling material plays an important role in the success of surgical endodontics. (Mattison et al., 1985) The depth of root end filling material should be 3 mm as more than that does not bestow any greater benefits whereas lesser depth may jeopardize the long-term success of apical seal. Hence, the depth of cavities in this study was kept at 3 mm. (Tidmarsh and Arrowsmith, 1989) Krakow et al. revealed that the bacterial microleakage technique was more accurate in leakage assessment, in comparison to dye penetration or radioisotopes techniques. (Saeed Moradi et al., 2015) Because of a lack of correlation between dye particles, isotopes and bacterial microleakage, the efficacy of root-end filling materials may be better determined using a bacterial microleakage model. (Adamo et al., 1999) So bacterial microleakage model was employed in this study. The results obtained in present study are similar to studies done by Estrela et al. who have compared the microbial leakage of various root end cements and MTA and found the evidence of microbial leakage on 3rd week in their 60 day experimental period. The result of our study in relation to Group A is not in accordance to the study done by Gish et al. They have also used an in vitro microbial leakage model using MTA as root end sealing material and concluded that MTA did not show any leakage throughout the experimental period (90 days). (Torabinejad et al., 1995) Currently there is dearth of study comparing bacterial microleakage on root end material Biodentine. Kokate S.R. & Pawar A.M. compared the microleakage using methylene blue dye and found that Biodentine exhibit lesser microleakage in comparison to MTA & GIC. Another comparative study done by Radeva et al. on bacterial microleakage showed the similar results that Biodentine was better than MTA. Shahjad Pathak did a comparative study on the evaluation of sealing ability of different root end filling materials with the help of Stereomicroscope and SEM and found least microleakage in Biodentine.

Conclusion

No material tested in this study was capable of providing a leak-proof seal. Although there is no evidence that an absolutely fluid-tight seal is necessary for clinical success, the question arises as to how much fluid movement is significant. Based on the results obtained from the present study, it was concluded that Biodentine exhibit best sealing ability than

mineral trioxide aggregate but statistically the difference between MTA and Biodentine was non-significant. Therefore the search for newer materials is aimed to reduce costs and to increase the feasibility of both professional clinician and patient.

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