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Comparison of Sealing Ability of White MTA, Gray MTA, Biodentine and Polymethylmethacrylate (PMMA) Bone Cement as Root-End Filling Materials - An *In Vitro* Study

Rakesh Mittal*, Meenu G Singla and Gitika Yadav

*Department of Conservative and Endodontics, Sudha Rustagi College of Dental Sciences and Research (SRCDSR), Faridabad, Haryana, India.

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ABSTRACT

Objective: This study compared the sealing ability of white MTA, gray MTA, PMMA bone cement and Biodentine as a root end filling material using a bacterial leakage model.

Materials and method: 150 single-rooted extracted teeth with straight roots were prepared with size #50/O6 K3XF files. The apical 3 mm of all the roots were sectioned at an angle 90 degrees to the long axis of the root using surgical straight plain fissure bur and a 3 mm deep root-end cavity was prepared in each sectioned root end by using ultrasonic retrotips. A tight fitting size #80 or #90 K-file (tip flattened with a bur) was positioned 3 mm short of the apical opening as a matrix against which the retrograde filling material was condensed in each prepared canal. Teeth were then randomly divided into four experimental groups (n=30) and two control groups (n=15). A fresh culture of *E fecalis* was introduced into the access cavity. Samples were incubated at 37°C in an incubator for the duration of experiment. The broth in the vials was monitored every day for color change for up to 28 days. A change in color from red to orange-yellow indicated that microbial leakage had occurred. Contaminated samples were plated on TSB agar plates to confirm the presence of *E. fecalis*. The number of samples which showed color change was calculated at 1st, 2nd, 3rd and 4th week.

Results: The difference between the frequencies of leakage among all the four study groups was found to be statistically significant ($p>0.05$) at 1st week, 2nd week and 3rd week but it was non-significant at the end of 4th week.

Conclusion: PMMA samples exhibited least microleakage followed by biodentine, gray MTA and white MTA. Under the experimental conditions, all the root end filling materials exhibited microleakage at the end of 4th week.

Keywords: Apical seal, Bacterial leakage model, MTA, Polymethylmethacrylate bone cement, Biodentine

INTRODUCTION

The goal of root canal treatment is the cleaning, shaping and complete obturation of the root canal system [1]. Penetration of microorganisms and their byproducts into filled root canal system causes failure in the treatment [2]. Endodontic surgery may be indicated in a number of cases or situations including: a strong possibility of failure from nonsurgical treatment; failure of nonsurgical treatment in which retreatment is either not possible or unlikely to result in a better outcome; excessive calcification of the root canal system; teeth restored with post and core crowns, iatrogenic shoulders or ledges, iatrogenic perforation of the canal [3].

The success of periapical surgery is dictated by elimination of infected tissues and adequate apical seal [4]. The procedure involves surgical debridement of pathological periradicular tissue, apical root-end resection, root-end cavity preparation and the placement of a root-end filling in an attempt to seal the root canal [5].

Throughout the dental history, a wide variety of materials that have been or are currently is being used as retrograde filling material. MTA was introduced in the year 1993 by Hirschberg et al. [6]. This material was originally indicated as a retrograde filling material for use in endodontic surgery and cases of intraradicular and furcal perforations. These indications of MTA are related to the possibility of use in

Corresponding author: Rakesh Mittal, Department of Conservative and Endodontics, Sudha Rustagi College of Dental Sciences and Research (SRCDSR), Faridabad, Haryana, India, Tel: 9813172742; E-mail: guddurohtak@gmail.com

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moist environments [6]. It is a material that has been extensively studied and has certain drawbacks such as difficulty in handling and very slow setting reaction, which might contribute to leakage, surface disintegration, loss of marginal adaptation and continuity of the material [7]. Earlier MTA was introduced with the gray color (GMTA) but it had some potential to discolor the overlying tissues. White MTA (WMTA) was therefore developed and introduced by Dentsply [8] in 2002 as an aesthetic alternative to GMTA.

Recently, newer materials biodentine has been introduced as root end filling materials. It was introduced in the year 2010. The material has indications similar to calcium silicate containing materials like MTA. Biodentine has better consistency, better handling and faster setting time that is 10-12 min [9], as compared to MTA which has a longer setting time of 2 h 45 min [7].

The first use of polymethylmethacrylate (PMMA) as a dental device was for the fabrication of complete denture bases. Its qualities of biocompatibility, reliability, relative ease of manipulation and low toxicity were soon seized upon and incorporated by many different medical specialties [10]. It has excellent adaptation to the cavity margins. The cement also tolerates a moist environment very well and is not affected by blood contamination [11].

In the literature, several methods have been used to assess the sealing ability of various root-end filling materials. The methods used include dye leakage, scanning electron microscopic evaluation, radioisotopes and bacterial leakage. Among these techniques the bacterial leakage model is considered as the most acceptable method [12].

The present study intended to compare the sealing ability of white MTA, gray MTA, PMMA bone cement and biodentine as a root end filling material using a bacterial leakage model.

MATERIALS AND METHODS

Selection of teeth

150 single-rooted extracted teeth with straight roots were selected for the study. Teeth with fractures, large restorations or gross caries, incomplete root formation and history of previous root canal therapy were excluded. All the teeth were stored in saline until used.

Preparation of teeth

During the preparation phase the experimental teeth were kept moist with 2" × 2" gauze sponges saturated with sterile saline. Endodontic access cavities were prepared with no. 2 round bur, pulp 'remnants' were extirpated with a fine barbed broach and the cervical third of each root canal system were flared using Gates Glidden burs Size #2 and #3. Working length of the canal in each tooth was measured using a # 10 K file (taking 1 mm short of actual apex) and confirmed using a radiograph. The root canal was instrumented to the master apical file size #50/O6 using a crown down technique with K3XF files. Teeth were irrigated with 5 ml of 5.25% sodium hypochlorite after each file size. A final 1 min rinse with 17% ethylenediamine tetraacetic acid (EDTA) followed by 5.25% NaOCl was done to remove the smear layer.

Root-end preparation

The apical 3 mm of all the roots were then sectioned at an angle 90 degrees to the long axis of the root using surgical straight plain fissure bur in a high speed hand piece. A 3 mm deep root-end cavity was prepared in each sectioned root end by using ultrasonic retrotips. A tight fitting size #80 or #90 K-file (tip flattened with a bur) was positioned 3 mm short of the apical opening as a matrix against which the retrograde filling material was condensed in each prepared canal. The prepared teeth were divided into four experimental groups with 30 teeth in each and two control groups with 15 teeth in each.

Group 1: White MTA (ProRoot MTA, Dentsply Tulsa Dental)

Group 2: Gray MTA (ProRoot MTA, Dentsply Tulsa Dental)

Group 3: Biodentine (Septodont)

Group 4: Polymethylmethacrylate (PMMA) bone cement

Group 5: Positive control; all the teeth in this group were not filled with any root end filling material after cavity preparation to act as positive controls.

Group 6: Negative control; the canals and root- end cavities were filled with sticky wax. All external surfaces were coated with two layers of nail polish including the sectional apical portion.

Bacterial leakage model

A modified version of the bacterial leakage model reported by Torabinejad et al. [13] was used. The external surfaces of the roots of teeth in Groups 1, 2, 3, 4 were coated with two layers of nail polish from the cementoenamel junction (CEJ) to 2 mm short of the prepared root apex. Glass vials were taken and holes were created in the center of rubber stoppers by heated instrument. Teeth were inserted under pressure into perforated rubber stopper and fixed to the level of cementoenamel junction. Entire model was sterilized in autoclave at 121°C, 15 lbs pressure for 20 min. After the sterilization procedure, the vials were filled with phenol red lactose broth (Becton, Dickinson and Company) and the caps were sealed to the vials so that the root ends were submerged in the broth. *Enterococcus fecalis* (ATCC 29212 strain) was grown overnight in 5 ml Trypticase soy broth (TSB) media to reach a final concentration of 1×10^9 colony forming units (CFU)/ml. Overnight culture (1 ml) was spun down and suspended in 1 ml of TSB media. A pipette

(Sigma-Aldrich) was used to introduce 10 µl of fresh *E. fecalis* culture into the access cavity. To ensure the viability of the bacteria and to avoid evaporation, the access cavity was flushed with saline every 48 h and a fresh culture of *E. fecalis* were introduced into the access cavity. Samples were incubated at 37°C in an incubator for the duration of experiment. The broth in the vials was monitored every day for color change for up to 28 days. A change in color from red to orange-yellow indicated that microbial leakage had occurred **(Figures 1a and 1b)**. Contaminated samples were plated on TSB agar plates to confirm the presence of *E. fecalis* **(Figure 1c)**. The number of samples which showed color change was calculated at 1st, 2nd, 3rd and 4th week.

Figure 1. a) Sample showing no change in color of broth indicating absence of microbial leakage; b) Sample showing change from red to yellow color indicating presence of microbial leakage; and c) Contaminated samples plated on TSB agar plates showing presence of *E. fecalis*.

The results were statistically analysed.

RESULTS

Statistical analysis

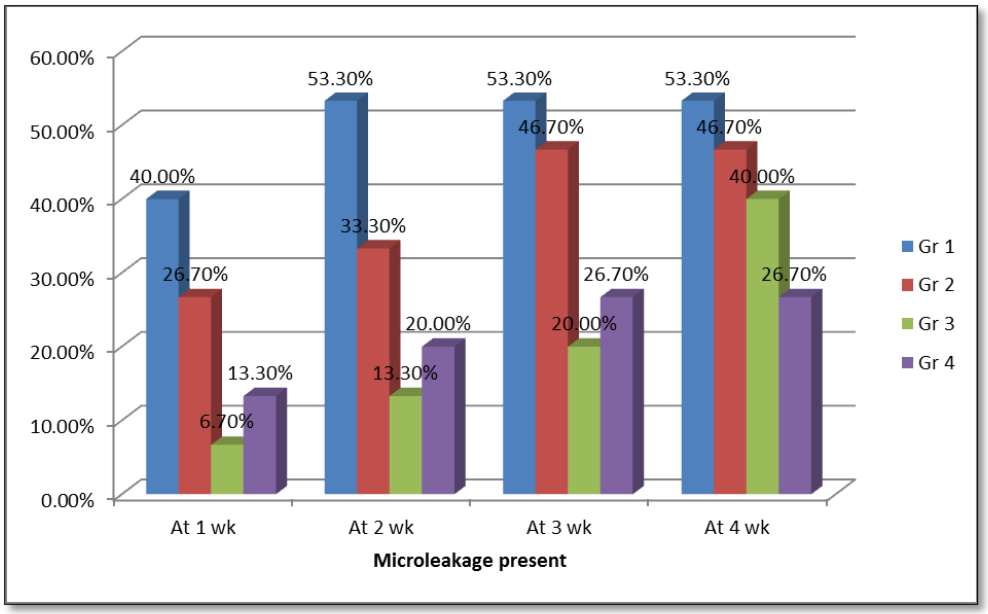
Data was entered into Microsoft excel sheet and analysed using SPSS version 21 (IBM). The outcome of the study (presence of leakage) was categorical in nature. Intergroup comparison of frequency of leakage was done using Chi square test. The level of statistical significance was set at 0.05. The graphs were prepared on Microsoft Excel **(Table 1)**.

Table 1. Intergroup comparison.

P value: Overall	**0.009, S**	**0.004, S**	**0.021, S**	0.187, NS
P value: gr 1*2	0.412,NS	0.192, NS	0.797, NS	0.797, NS
P value: gr 1*3	**0.005, S**	**0.002, S**	**0.015, S**	0.438, NS
P value: gr 1*4	**0.039, S**	**0.015, S**	0.064, NS	0.064, NS
P value: gr 2*3	**0.028, S**	0.125, NS	0.054, NS	0.795, NS
P value: gr 2*4	0.333, NS	0.382, NS	0.180, NS	0.180, NS
P value: gr 3*4	0.671, NS	0.731, NS	0.761, NS	0.412, NS

Results show that all the root end filling materials exhibited microleakage. None of the negative control groups showed any color change indicating no leakage and all of the positive controls demonstrated a color change indicating leakage within the first 24 h.

The numbers of samples of experimental groups with bacterial leakage at the end of 1st, 2nd, 3rd and 4th week are listed in **Graph 1**.



Graph 1. Bacterial leakage at the end of 1st, 2nd, 3rd and 4th week.

The difference between the frequencies of leakage among all the four study groups was found to be statistically significant ($p>0.05$) at 1st week, 2nd week and 3rd week but it was non-significant at the end of 4th week.

On inter group comparison, difference between group 1 and group 3 was statistically significant at 1st week, 2nd week and 3rd week. While between group 1 and 4 difference between the frequencies of leakage was significant at 1st week and 2nd week. On comparison between group 2 and 3, this difference was significant only at the end of 1st week. At the end of 4th week, there was no significant difference in any of the groups regarding frequency of microleakage.

DISCUSSION

The purpose of placing a retrograde seal after apicoectomy is to establish an effective barrier between the root canal and the periapical tissues. The apical seal is the single most important factor in achieving success in surgical endodontic.

A plethora of materials have been suggested for use as root end filling and have been tested for their sealing ability. Among the various root end filling materials tested, Mineral Trioxide Aggregate (MTA) has shown good sealing ability and biocompatibility in previous *in vitro* and *in vivo* studies [13]. The powder particles are hydrophilic and small. When they come in contact with the moisture the hydration reaction occurs that results in colloidal gel structure which solidifies in the mineralized tooth structure [14]. The main characteristic of white MTA was the near-elimination of iron from the original formulation, depleting the set MTA of aluminoferrite, which was responsible for the gray coloration. This color was problematic in some circumstances where the cosmetic appearance of the treated tooth was affected adversely.

Biodentine is a new calcium silicate-based root end filling material which uses novel active biosilicate technology to ensure superior mechanical properties [14]. In addition to the chemical composition based on the Ca_3SiO_5 and water chemistry which brings the high biocompatibility of already known endodontic repair cements like MTA, it has increased physico-chemical properties like short setting time, high mechanical strength which make it easy to handle and compatible, not only with classical endodontic procedures, but also for restorative cases of dentine replacement [15].

The search for alternative materials is aimed to reduce costs and to increase the feasibility to both professional and patient. PMMA is a new material that might potentially provide the necessary properties of a root end filling material. PMMA bone cement has been widely used in orthopedic surgery [7].

As there is limited literature for PMMA as a retrograde filling material the aim of this study was to compare its sealing ability with gray MTA, white MTA and biodentine as a root end filling material by using bacterial leakage model.

A successful periapical surgery requires appropriate root-end resection, preparation and adequate apical seal. Apical root-end resection of 3 mm at 90° angulation was done in our study to reduce 98% of the apical ramifications and 93% of the lateral canals, which might be responsible for endodontic failure [16]. 90° plane of sectioning was selected for this study as it has been proved to be most acceptable by earlier studies. The inclined plane sectioning at 30 or 45° angle could have disadvantages like open dentinal tubules, errors in post-operative radiographs, more mechanical stresses, loss

of dentin, cementum and bone that could result in compromised healing [15].

Traditionally, the root-end cavity is prepared with burs used with low-speed hand piece. Cavity preparation with burs has some disadvantages including limited operative field and root-end bevel, which increases the number of exposed dentinal tubules on the root-end surface. In the present study, ultrasonic surgical tips were used as an alternative to the burs for root-end cavity preparation. It allows cleaner and deeper cavity centered in the root canal and reduced bevel angle thus having the advantage of decreased number of exposed dentinal tubules at the resected surface and so the microleakage is also minimized [17].

In literature, several *in vitro* methods have been used to assess the sealing ability of root-end materials: dye penetration, radioisotopes, bacterial leakage studies, electrochemical techniques, scanning electron microscopy, and fluid filtration methods [1]. Among these techniques the bacterial leakage model seems to be the most clinically relevant method [6]. Thus, it was used in the present study to evaluate microleakage *in vitro*. It has been argued in the case of dyes, if the size of the dye molecules is small enough, a false positive result may be obtained. In contrast, bacteria would be a better tracer, especially when testing leakage of hydrophilic materials. Moreover leakage tests involving microbiology are more technically exacting. The bacterium selected as the leakage marker should preferably be an endodontic pathogen, and easy to cultivate [18]. *Enterococcus fecalis* was chosen as the bacteriological marker in this study It is commonly found in a high percentage of root canal failures and is able to survive in the root canal as a single organism or as a major component of the flora, while other bacteria commonly associated with endodontic infections may require symbiotic support from other bacteria [2,12].

Under these experimental conditions, all the root end filling materials exhibited microleakage. PMMA samples exhibited least microleakage (26.7%) followed by biodentine (40.0%), gray MTA (46.7%) and white MTA (53.3%) after 4 weeks. It was noted that biodentine exhibited least microleakage up to 3 weeks but at the end of 4th week PMMA showed least microleakage. Although the difference in results seems to be clinically relevant but they did not reach the level of statistical significance.

Though statistically not significant, gray MTA showed less bacterial micro-leakage in comparison to white MTA in the present study. Aranha et al. [19], Matt et al. [20], Al-Hezaimi et al. [21] and Ferris and Baumgartner [22] showed more leakage with WMTA compared with GMTA, although the differences between the two materials in these studies were not statistically significant, perhaps the small differences were caused by differences in setting expansion of GMTA and WMTA. Kazem et al. [2] found no significant difference between gray MTA and white MTA in terms of bacterial leakage. Their results are consistent with the present study.

In the present study, the sealing ability of biodentine was found better than both gray and white MTA in early stages. Findings of our study were consistent with the results of Ravichandra et al. [12] and Kokate and Pawar [15]. A study conducted by Han and Okiji [23] demonstrated that Biodentine has more prominent biomineralization ability than MTA, with wider calcium and silicon rich layer at material-dentine interface. Soundappan et al. [16] and Mandava et al. [14] observed different results in their respective studies which showed that the microleakage of MTA was comparatively less than Biodentine. The disparity in results of these two studies may be attributable to differences in the methodology employed. Soundappan et al evaluated the marginal adaptation of Biodentine in comparison with Mineral Trioxide Aggregate (MTA) as a root end filling material, using Scanning electron microscopy (SEM) and Mandava et al. [14] evaluated the apical microleakage of Mineral trioxide aggregate and Biodentine using dye penetration under stereomicroscope whereas in the present study, sealing ability of Biodentine and MTA was evaluated using bacterial leakage model.

In the present study PMMA bone cement has shown the better sealing ability than white MTA in the early stages while it showed comparable results with gray MTA, white MTA (later stage) and biodentine. Similar study by Girish et al. [7] comparing PMMA bone cement with other root end filling materials showed that PMMA bone cement exhibited less microleakage when compared with calcium phosphate cement and MTA.

The current study is an attempt to evaluate the use of polymethylmethacrylate bone cement in the field of endodontic where the search for the ideal repair material is a continuing process. Further research is required to assess the bioactive property of the bone cement especially the longevity of calcium release and long term stability in relation to body tissues and tissue fluids after its placement.

CONCLUSION

Within the limitations of this study, it can be concluded that the seal provided by PMMA bone cement as a root end filling material was comparable to MTA and biodentine. It had better working properties, which could overcome potential disadvantages faced with MTA. PMMA bone cement thus seems to be an excellent and promising economical material for root end filling.

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