Journal of Chitwan Medical College 2021;11(38):41-45
Available online at: www.jcmc.com.np

ORIGINAL RESEARCH ARTICLE

MICROLEAKAGE IN MINERAL TRIOXIDE AGGREGATE AND BIODENTINE AS ORTHOGRADE APICAL PLUGS IN PERMANENT TEETH SIMULATED WITH OPEN APICES: AN IN-VITRO EVALUATION

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Received: 29 Nov, 2021
Accepted: 16 Dec, 2021
Published: 25 Dec, 2021

Key words: Apexification; BiodentineTM; Blunderbuss canals; Dye penetration method; MTA AngelusR.

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*DOI:*https://doi.org/10.54530/jcmc.581

Citation

Thapaliya B, Koul MV, Upadhyay VK, Khare A. Microleakage in mineral trioxide aggregate and biodentine as orthograde apical plugs in permanent teeth simulated with open apices: an in-vitro evaluation.Journal of Chitwan Medical College.2021;11(38):41-5.

Peer Reviewed

ABSTRACT

Background: The divergent apical architecture makes complete debridement and holding of the obturating material within the root canal impossible as no apical barrier exists, which may result in a canal that is susceptible to leakage. The aim of the study was to evaluate the apical microleakage of MTA Angelus[R] and Biodentine[TM] when used as orthograde apical plugs.

Methods: Sixty extracted sound human anterior teeth simulated with open apices were equally distributed in two groups viz group A [MTA Angelus[R] (Mfd by Angelus, Brazil)] and group B [Biodentine[TM] (Mfd by Septodont, France)]. Apical microleakage in both groups was evaluated using dye penetration method. Dye penetration data of two independent groups were summarized as Mean ± SE (standard error of the mean) and compared by independent Student's t test. A two-tailed (α=2) $p<0.05$ was considered statistically significant.

Results: Apical microleakage of MTA Angelus[R] [Group A] ranged from 1.30 to 3.10 mm with mean (± SE) 2.16 ± 0.10 mm and median 2.05 mm whereas in Biodentine[TM] [Group B] it ranged from 0.20 to 1.20 mm with mean (± SE) 0.68 ± 0.06 mm and median 0.60 mm. Sealing ability of Group B (Biodentine[TM]) was significantly better ($p<0.001$) and 68.5% higher than Group A (MTA Angelus[R]).

Conclusions: Both the materials exhibited microleakage. There was a significant difference ($P<0.001$) between the two groups and Biodentine[TM] showed to have less apical microleakage than MTA Angelus[R].

INTRODUCTION

The divergent apical architecture makes complete debridement and holding of the obturating material within the root canal impossible resulting in a canal that is susceptible to leakage.[1] An additional problem with immature teeth are thin and fragile root canal walls which are prone to fracture during and after treatment.[2] Having known that calcium hydroxide is one of the most sought therapeutic agents still used till date, however, it exhibits some drawbacks like, multiple appointments, uncertainties in apical closure and susceptibility to coronal microleakage. An alternative for the multi appointment apexification procedure is one visit technique using an apical barrier for which MTA has been suggested.[3]

Torabinejad et al introduced MTA in 1993. MTA provides scaffolding for the formation of hard tissue and the potential of a better biological seal.[4] MTA is indicated for creating an apical plug during apexification.[5] Although this material provides remarkable physical and biological properties its use has always remained a challenge due to its technique sensitivity and prolonged setting time.[6] Taking into consideration its drawbacks, another calcium silicate –based bioactive restorative cement was discovered under the name of Biodentine[TM].[6] This material is actually formulated using the MTA based cement technology to achieve a higher degree of effectiveness in its physical qualities and handling characteristics.[7] Dye extraction or dissolution method, bacteria and toxin infiltration method, air pressure method , dye penetration, fluid filtration are various methodologies that aids assessment of microleakage.[8] This in-vitro study was carried out to evaluate the apical microleakage of MTA Angelus[R] and Biodentine[TM].

METHODS

The study was conducted in the department of Paedodontics and Preventive Dentistry, and department of Oral Pathology of Career Post Graduate Institute of Dental Sciences and Hospital, Lucknow, India. The study was approved by the ethical committee of Career Post Graduate Institute of Dental Sciences and Hospital, Lucknow, India and the permission to carry the study was obtained.

Freshly extracted sound human (seventy) single rooted perma-

nent anterior tooth with closed apices were freed of tissues and blood by cleaning them under running tap water. It was disinfected for an hour with the specimen being immersed in 3% sodium hypochlorite [NaOCl (PrevestDenPro, India)] followed by scaling, and eventually placing it in artificial saliva (Wet mouth, ICPA health product. Ltd) at room temperature until use. The collected samples were then examined under stereomicroscope at 10 × magnification to evaluate cracks, fractures and external root resorption.

Out of seventy collected samples, ten were discarded due to the presence of cracks and fractures. Then in all, sixty samples were left and used in the study. Teeth were equally distributed in two groups viz group A [MTA Angelus^{R} (MTA Angelus^{R}, Mfd by Angelus, Brasil)] and group B [Biodentine™ (Biodentine™, Mfd by Septodont, France)]. In each group, the root apices of the selected samples [n=30] were removed by using a diamond disc (Kerr, USA) under water coolant 2 mms from the apical root ends to simulate the open apices (shown in figure 1).

Access cavity was prepared in each sample with aid of airrotor handpiece (NSK, Japan) and endo access bur (Dentsply, Switzerland). The working length of canal was determined by using ISO 15 No k-file (Dentsply, Switzerland) into the canals, until the instrument tip was visible at the cut end of the apices. Biomechanical preparation of root canals was done upto ISO 80 k-file (Dentsply, Switzerland) with the step- back technique. 17% EDTA (Mixodent, India) and 3% sodium hypochlorite [NaOCl] (PrevestDenPro, India) were used alternatively during biomechanical preparation. A final flush with normal saline (Krpl, India) solution was performed and canals were dried with the help of absorbant paper points (Perl Dent, Vietnam).

MTA Angelus^{R} and Biodentine™ were mixed according to manufacturer's instructions. Apical plugs of 4mms with MTA Angelus^{R} and Biodentine™ in each tooth of group A and B was made. After 15 minutes in group A and 12 minutes in group B the remaining canal space was obturated with gutta-percha (Pearl Dent,Vietnam) and root canal sealer i.e Endoflux (Ammdent,India) using single cone technique, after which the coronal access cavity was sealed with a glass ionomer cement (GC, Gold Label 2,Japan). After sealing the coronal cavities of two groups, A and B the external root surfaces were completely covered with 2 coats of nail polish (Coloressence, New Delhi), except for an area of 2.0mm around the cut ends and allowed to dry.

The specimens were then immersed in the solution of 1% methylene dye (N K Sales Corporation) for 48hours. After 48 hours specimens were washed in running tap water for 5 minutes and mounted on the acrylic blocks made with cold cure acrylic (DPI-RR Cold Cure,India) to facilitate longitudinal splitting of the specimens using diamond disc (Kerr, USA) under water coolant. One half of the each sectioned tooth was examined for dye penetration under the Stereomicroscope (Labomed, USA) with occular micrometer [(Erma, Japan), shown in figure 2 and 3] whereas the other half was discarded. Occular micrometer is a linear scale of 10mms having 100 divisions where each division is equal to 0.1mm. The dye penetration of each sample of both groups A and B was recorded in millimeters. The data thus obtained was tabulated and subjected to statistical analysis using Student's t test. A two-tailed (α=2) $p<0.05$ was considered statistically significant. Analysis was performed on SPSS software (Windows version 17.0).

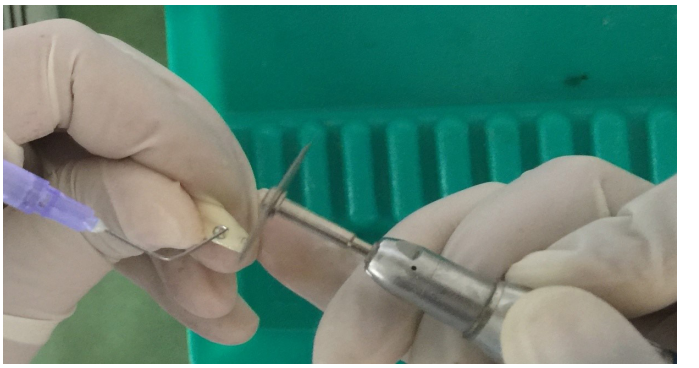

Figure 1: Removing root apex

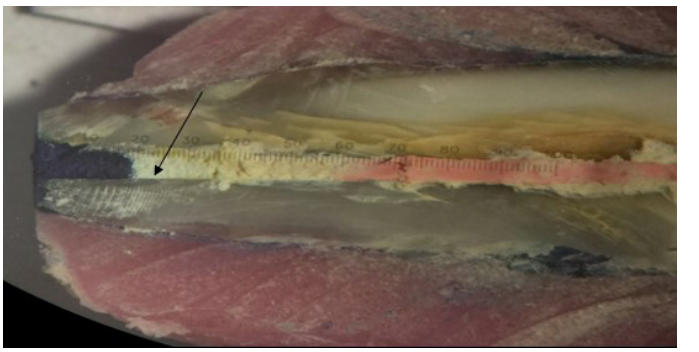

Figure 2: Dye penetration in MTA Angelus^{R} apical plug under Stereomicroscope

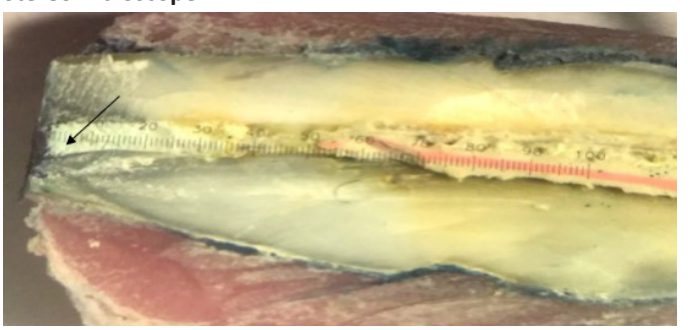

Figure 3: Dye penetration in Biodentine™ apical plug under Stereomicroscope

RESULTS

The observed dye penetration of two groups is summarized (Minimum, Maximum, Mean, SD, SE and Median) in Table 1. The dye penetration of MTA Angelus^{R} [Group A] ranged from 1.30 to 3.10 mm with mean (± SE) 2.16 ± 0.10 mm and median 2.05 mm whereas in Biodentine™ [Group B] it ranged from 0.20 to 1.20 mm with mean (± SE) 0.68 ± 0.06 mm and median 0.60 mm. The mean dye penetration of Biodentine™ lowered comparatively as compared to MTA Angelus^{R} (MTA Angelus^{R} >Biodentine™). Comparing the mean dye penetration of

two groups, Student's t test showed significantly different and lower (68.5%) dye penetration in Biodentine™ i.e Group B as compared to MTA Angelus® i.e Group A (2.16 ± 0.10 vs. 0.68 ± 0.06, mean difference=1.48 ± 0.12, 95% CI of difference=1.24 to 1.71, t=12.69, p<0.001). In conclusion apical microleakage in Biodentine was significantly less thereby indicating a better sealing ability [p<0.001] than MTA Angelus® which is shown in the form of bar diagram in figure 4.

Table 1: Summary statistics of dye penetration in millimeters (mm) of two groups A and B

Summary statistics	Group A [MTA Angelus®]	Group B [Biodentine™]
N	30	30
Minimum	1.30	0.20
Maximum	3.10	1.20
Mean	2.16	0.68
SD	0.55	0.33
SE	0.10	0.06
Median	2.05	0.60

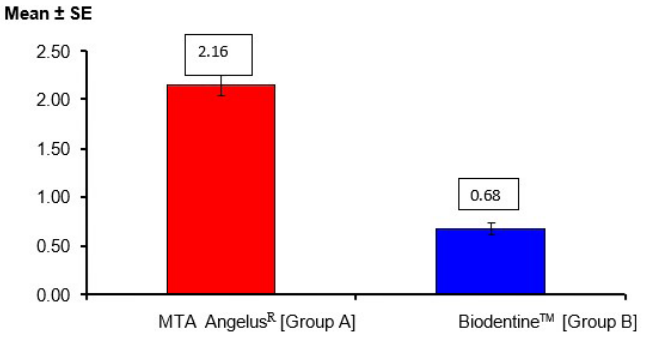

Figure 4: Bar diagram showing comparison of mean dye penetration between two groups A and B

p<0.001- as compared to Group A [MTA Angelus®]

DISCUSSION

The advancement of restorative materials and techniques continues to enhance the clinical success of numerous restorative procedures. Despite these new innovations microleakage persists as one of the main causes of restoration failure. Microleakage is the movement of bacteria, fluids, molecules, and/or ions between the tooth and restoration margins.[9] Microleakage is a result of the external environment invasion through the margins of the restoration which also can occur internally.[10,11] Microleakage can cause a variety of adverse effects, such as secondary caries, higher sensitivity of the restored tooth and interfacial staining leading to pulp pathology.[12,13] Leakage tests can be subdivided into old and contemporary methods. Old methods were used to test the presence of gaps and the sealing ability of different restorative materials. Some of these methods include air pressure, fluid filtration, electrochemistry, neutron activation, bacteria and artificial caries.[14]

However, these techniques were found to be non-representative of leakage and thus have been replaced by more contemporary methods such as radioisotope method, acetate peel technique, dye penetration, optical coherence tomography, microcomputed tomography and confocal laser scanning microscopy.[15] The main objective of all endodontic procedures is to obtain a hermetic seal between the periodontium and root canal foramina.[16] The apical plug method is regarded as the golden standard for apexification as it is a single visit technique, has less need for patient's cooperation and gives satisfactory results [17] therefore, MTA and Biodentine are recommended options to perform apexification plugs.[18]

In-vitro experimental study should be performed before embarking on to in-vivo studies.[19] In vitro studies provide us with the platform to create, compare and check dental materials prior to their clinical application. The present study was thus designed to be an in-vitro one and aimed to evaluate the apical microleakage of MTA Angelus® and Biodentine™ when used as orthograde apical plugs and compare their sealing ability using dye penetration method. The staining of microleakage and nanoleakage using colored agents is the most commonly used technique. Dye penetration method involves the use of contrasting dyes as an immersion solution to stain the areas of microleakage.[15] Using dyes is a simple, safe and most commonly used method to observe microleakage which can be done easily without any chemical reaction.[15]

In the present study microleakage was assessed by immersing samples in a solution of 1% methylene blue dye for 48 hours. 1% methylene blue dye solution was used in this study because its molecule size is very small, even smaller than bacteria; thus, methylene blue 1% solution can penetrate farther than other dyes because of its small molecular size (0.5-0.7nm).[20] This in-vitro experimental model consisted of extracted mature human anterior teeth that were resected 2mms from the apical root ends to simulate immature roots with open apices. This model produces standardized and reproducible anatomy that duplicates the essential features of a blunderbuss apex. Hachmeister et al and Mehmet Bani et al also resected the apices of extracted teeth in their studies.[21,3] Orthograde apical plugs of 4 mms were made in both groups of MTA Angelus® and Biodentine™ following manufacturers' instructions. The result of 4mms thick apical plugs have been demonstrated with respect to root canal sealing ability and resistance to displacement.[22]

In the present study 15 minutes in MTA Angelus® group and 12 minutes in Biodentine™ group was awaited for obturation because setting time of MTA Angelus® is 15 minutes and that of Biodentine™ is 12 minutes as suggested by manufacturer. After sealing the coronal cavities of both groups external root surfaces were completely covered with 2 coats of nail polish except for an area of 2mms around the cut ends so that dye can penetrate only through the apical plugs making other surfaces impermeable. Longitudinal sectioning method was used to assess the dye penetration into the filling material._Schäfer and Olthoff have shown that greater linear dye penetration provides enough data regarding apical leakage even though

it does not provide data about area.[23] In the present study Student's t test showed significantly different and lower (68.5%) dye penetration in Biodentine™ as compared to MTA Angelus® (2.16 ± 0.10 vs. 0.68 ± 0.06, mean difference=1.48 ± 0.12, 95% CI of difference=1.24 to 1.71, t=12.69, p<0.001).

Ankita Khandewal et al conducted an in-vitro study where sealing ability of MTA and Biodentine™ was compared using 0.5% rhodamine dye and leakage was evaluated under confocal laser scanning microscope which showed Biodentine™ to have better sealing ability than MTA which was statistically significant.[24] Sakshi Malhotra and Mithra N Hegde evaluated the marginal sealing ability of ProRoot MTA, MTA Angelus®, Biodentine™ and GIC using dye penetration method where the result of the study showed that apical microleakage was present in all the samples but least amount of apical microleakage was seen with Biodentine™ and the difference was statistically significant.[25] Allwyn Samuel et al compared the sealing ability of MTA and Biodentine™ where the extent of marginal adaptation of experimental materials was measured by SEM. Result showed lesser microleakage in Biodentine™ compared to MTA and the difference was statistically significant (P< 0.01).[26] Elka Radeval et al compared the microleakage of MTA and Biodentine™ using dye penetration method and showed higher difference in mathematical value for MTA but the difference was statistically insignificant (P 0.05).[27] Mehmet Bani et al carried out an in vitro study to evaluate the apical microleakage of Biodentine™ and MTA by the fluid filtration technique where the apical sealing ability of Biodentine™ was comparable to MTA[3] which is in agreement with the studies done by Torabinejad et al, Verissimo et al, de Vasconcelos et al and Garip et al.[28,29,30,31] Abimanyu et al evaluated the microleakage of MTA and Biodentine™ using 1% methylene blue dye under stereomicroscope where the result of the study showed insignificant difference in microleakage between MTA and Biodentine™.[20] Gizem Ozbay et al assessed the sealing ability of Biodentine™ and White MTA using fluid Infiltration Technique. It was concluded that MTA showed less microleakage when compared with Biodentine™ and difference was statistically significant (p< 0.05).[32]

Results of the present study revealed that samples filled with Biodentine™ showed least microleakage value thereby indicating better sealing ability when compared to MTA Angelus®. The probable reasons of this could be :

a.Biodentine is found to be associated with high pH (12) and releases calcium and silicon ions which stimulates mineralization and create "mineral infiltration zone" along dentin-cement interface imparting a better seal.[33]

b. The sealing ability of Biodentine™ is most likely through the formation of tags. Han and Okiji showed that calcium and silicon ion uptake into the dentine leading the formation of tag like structures in Biodentine™ was higher than MTA.[33]

c. Biodentine™ has an advantage of fast setting i.e 12 minutes due to the addition of calcium chloride which acts as an accelerator as compared to MTA Angelus® i.e 15 minutes, thereby sealing the interface earlier to avoid further leakage.[24]

d. Porosity and pore volume in set Biodentine ™ is also less than MTA indicating a better sealing ability.[24]

e. The creamy consistency of Biodentine™ after mixing as compared to a homogenous wet sand consistency of MTA Angelus® could be one of the reasons of improved handling properties and a better seal with Biodentine™.

f. Although tricalcium silicate appears to be a common ingredient in both MTA and Biodentine™, x-ray diffractometry of unhydrated cements revealed that Biodentine™ consisted of triclinic form of tricalcium silicate while MTA consisted of the monoclinic form.[34] Another difference would be the finer particle size of tricalcium silicate in Biodentine™ as shown by the greater value of specific surface area of Biodentine™ (2.811m2 /g) in comparison to that of MTA (1.0335m2 /g). The smaller particle size of Biodentine™ could be another reason for its better sealing and less microleakage.[34] Ending this study it was deduced that Biodentine™ showed less microleakage than MTA Angelus®.

CONCLUSION

Based on the observations of this in-vitro study it was concluded that both the materials MTA Angelus® and Biodentine™ when used as apical plugs in open apices teeth exhibited microleakage. There was a significant difference (P< 0.001) between the two groups, and Biodentine™ showed to have less apical microleakage than MTA Angelus®; thereby indicating a better sealing ability. Thus, Biodentine™ is superior to MTA Angelus® and can be a better option in single sitting apexification technique to create an artificial apical barrier in which obturation can be compacted.

CONFLICT OF INTEREST: None

FINANCIAL DISCLOSURE: None

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