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Evaluation of the sealing ability of mineral trioxide aggregate repair HP, biodentine and white mineral trioxide aggregate as furcation repair materials: An in-vitro ultraviolet-spectrophotometric analysis

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***Abstract*---**This study aims to evaluate the sealing ability of Mineral Trioxide Aggregate Repair HP, Biodentine and White Mineral Trioxide Aggregate(WMTA) as furcation repair materials by using Ultraviolet-Spectrophotometric analysis. A total of 80 freshly extracted human permanent mandibular first and second molar teeth were included. Teeth were decoronated 3mm above the cemento-enamel junction and roots were amputated 3mm below the furcation. Endodontic access cavity opening was done and root canal orifices were sealed with sticky wax. Artificial perforations were made in furcation areas of teeth using round bur.80 specimens were divided into 3 Experimental groups with 20 specimens per group; Group A (MTA Repair HP), Group B (Biodentine), Group C (WMTA) used as furcation repair materials and the remaining 20 specimens were equally divided between Positive control and Negative control groups. Specimens were immersed in Indian ink dye for 48hours and were then subjected to dye extraction method by completely dissolving the teeth in 65% nitric acid and the obtained solutions were analysed using Ultraviolet-Spectrophotometer and readings were recorded as absorbance units. Obtained data was statistically analysed using One-way ANOVA and Tukey's-Post hoc tests. No statistically significant difference was seen in the sealing ability between MTA Repair HP and Biodentineas furcation repair materials.MTA Repair-HP showed the best sealing ability followed by Biodentine. White MTA showed poor sealing ability.

***Keywords*---**absorbance units, biodentine, MTA Repair HP, sealing ability, white MTA.

Introduction

Root canal treatment or endodontic therapy helps in achieving the integrity of natural dentition by maintaining its form, function and esthetics. Ingle reported that perforations were the second greatest cause accounting for 9.6% of endodontic failures(Ford *et al.*, 1995). American Association of Endodontist's (AAE's) Glossary of Endodontic terms defines; Perforation as a mechanical or pathological communication between the root canal system and the external tooth surface (American Association of Endodontists., 2003; Hamad*et al.*, 2006). Perforations can occur due to dental caries, resorption or procedural accidents during endodontic access cavity preparations, shaping of root canals and post-space preparation affecting the prognosis of root canal treatment(Nishtha*et al.*,2014).

Regardless of the underlying cause, Furcation perforation is followed by bacterial contamination, periodontal fibers destruction, periradicular tissue injury, inflammation, bone resorption and shows the worst possible outcome in root canal treatment (Hamad*et al.,*2006).Sinai found that the prognosis of tooth with afurcal perforation depends on time for which the perforation site is open to contamination and its repair as quickly as possible with a biocompatible endodontic restorative cement(Sinai.,1977).The ideal properties of a furcal perforation repair material are; It should be non-toxic, non-corrosive, nonstaining, have bactericidal or bacteriostatic properties, easy to manipulate, sets rapidly, well tolerated by periradicular tissues, promote regeneration of periradicular tissues, dimensionally stable, radiopaque and remain unaffected by the presence of moisture and low pH levels (Alhadainy*et al.,* 1993).

The most preferred furcation repair materials in endodontics are bioactive materials like Mineral Trioxide Aggregate(MTA) and Biodentine. Biodentine is a Tri-Calcium silicate based restorative cement. It contains calcium chloride as a setting accelerator. It bonds with the tooth dentin in chemo-mechanical way. It form stag-like structures at its interface with the tooth structure, as calcium silicate degrades the collagenous component of the interfacial dentine (Han *et al.,* 2011; Grover *et al.,* 2020).

MTA is basically available as Gray MTA and White MTA. MTA is mainly composed of calcium oxide, dicalcium silicate, tricalcium silicate, tricalcium aluminate, tetracalciumaluminoferrite and calcium sulphate dihydrate. However, White MTA lacks tetracalciumaluminoferrite. Gray MTA causes discolouration to tooth and gingiva. The absence of Iron oxide in White MTA causes the colour change from Gray to White (Torabinejad*et al.,* 1995).The setting time of MTA is approximately 3-4 hours. During the initial stages of its setting, the pH is 10.2 and upon its final setting it is 12.5. MTA has several drawbacks such as prolonged setting time, difficult handling characteristics, blood contamination had adverse effects on the retention characteristics of MTA with the tooth structure and this seems to be aggravated when used as furcation repair material (Rahimi*et al.,* 2013).

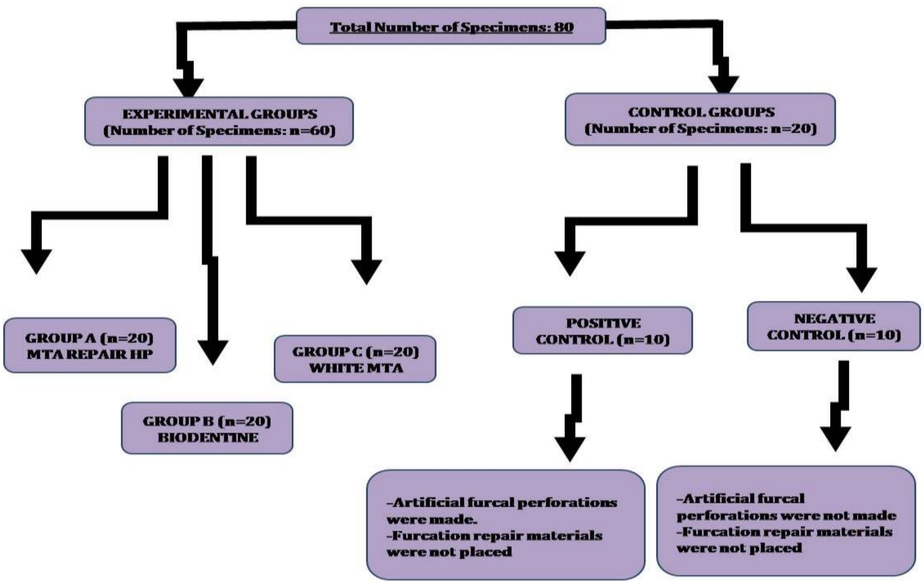
Recently, a new formulation of MTA was introduced; Mineral Trioxide Aggregate (MTA) Repair HP, is an endodontic restorative cement with High Plasticity (HP) consisting of mineral oxides in the form of fine hydrophilic particles. The composition of MTA Repair HP is very much similar to White MTA, except the liquid in MTA Repair HP contains plasticizer along with water and powder contains calcium tungstate as radiopacifier instead of bismuth oxide (Mirona*et al.,* 2021). There is not much data to determine the sealing ability of MTA Repair HP as furcation repair material in endodontics. So, this study aims to evaluate the sealing ability of MTA Repair HP, Biodentine and White Mineral Trioxide Aggregateas furcation repair materials using Ultraviolet-Spectrophotometric analysis.

Materials and Methods

Eighty freshly extracted human permanent mandibular first and second molar teeth were collected in Triveni Institute of Dental Sciences, Hospital and Research centre, Bilaspur. India. Inclusion criteria: Non-carious, non-fractured, un-restored, closed root apices, divergent roots. Exclusion criteria: Carious, fractured, restored, open root apices, fused roots. All the teeth were cleaned of superficial debris, calculus, residual tissue tags with ultrasonic instruments and immersed for 30 minutes in 3% sodium hypochlorite solution for its disinfection, washed with tap water and were then stored in 0.5% thymol at room temperature until used. To facilitate manipulation, all the teeth were decoronated 3mm coronal to the cemento-enamel junction and the roots were amputated 3mm apical to the furcation area using a diamond disk mounted on a contra-angled latch type handpiece (NSK, Japan). A standardized endodontic access cavity preparation was made in each molar tooth using Endo access bur No. 2 (Dentsply Maillefer, Switzerland) in a high speed conta-angled airoter handpiece (NSK, Japan) and the root canal orifices were located (Figure 1). The contents of the pulp chamber and root canals were removed with a spoon excavator and barbed broaches (DentsplyMaillefer, Switzerland).In all the specimens, to ensure each furcal perforation was centered exactly between the roots of the tooth, a black marker pen was used to mark the location on the pulpal floor of the access cavity. All the specimens were then divided into groups (Figure 2).

Figure 1: Endodontic access cavity preparation

Figure 2: Experimental groups and Control groups



In all the specimens; except in the Negative control group, Artificial furcal perforations were made of 2mm in diameter with a No. 2 round carbide bur (DentsplyMaillefer, Switzerland) in a high speed contra-angled airoter hand piece (NSK, Japan) from the coronal endodontic access of teeth (Figure 3).The depth of furcal perforations depended on the dentin-cementum thickness from the pulpal floor to the furcation area. The height of walls in all the artificially created furcal perforations was 2mm as measured by using William's graduated periodontal probe, so as to standardize the thickness of the three furcation repair materials to 2mm and in teeth with height of walls of perforations less than 2mm were excluded from the study. In all the specimens, root canal orifices and the sectioned root apices were sealed with Sticky wax (Figure 4), except in the artificially made furcal perforations.

Manipulation of MTA Repair HP:- 0.085 grams of powder was mixed with 0.25ml of liquid (1 drop) using a plastic spatula on a non-absorbent paper pad following manufacturer's instructions to obtain putty like consistency and was carried into the prepared furcal perforation site using a messing carrier(AMT Dental Pvt. Ltd. Navi Mumbai) and gently condensed with finger pluggers (Mani, Japan) upto 2mm thickness (Figure 4; Figure 5).

Manipulation of Biodentine:-0.4 grams of powder was mixed with 0.18ml of liquid (5 drops) using a plastic spatula on a non-absorbent paper pad following manufacturer's instructions into a homogenous mass and was carried into the prepared furcal perforation site using an amalgam carrier (GDC, Mumbai) and gently condensed with finger pluggersupto2mm thickness (Figure 4; Figure 6). Manipulation of White MTA:0.14 grams of powder was mixed with 1.5ml of liquid (3 drops) in 3:1 ratio using a plastic spatula on a non-absorbent paper pad following manufacturer's instructions into a packable consistency and was carried into the prepared furcal perforation site using a messing carrier and gently condensed with finger pluggers upto 2mm thickness (Figure 4; Figure 7).

In all the Experimental & Positive control group specimens, teeth were fully coated with two layers of nail varnish except over and1mm around the furcation repair materials both from external and internal surfaces of the tooth structure. In Negative control group specimens, teeth were fully coated with nail varnish except over and 1mm around the marked furcal perforation sites. All the specimens were then stored at 37^{0}C in 100% relative humidity for 48hours to allow the furcation repair materials to completely set. Then the specimens of each group were placed in separate petri dishes containing Indian ink dye (Himedia Laboratories Pvt. Ltd. Mumbai, India) such that all the specimens were completely immersed in the dye for 48 hours to allow for adequate dye absorbance. After removal from the dye, teeth were rinsed under running tap water for 10 minutes and nail varnish was completely removed with a scalpel.

Each tooth was then placed in separate test tubes containing 2ml of concentrated 65% nitric acid (Thermofisher Scientific Pvt. Ltd. Mumbai, India) for 3 days to denature and completely dissolve the teeth (Dye extraction method). The solutions thus obtained were transferred into Eppendorf tubes and centrifuged (Sorvall Legend X1R Centrifuge, Thermo Scientific Pvt. Ltd. India) at 3500 rpm (revolutions per minute) for 5 minutes. From each sample, 2ml of the supernatant layer was collected and transferred into plastic cuvettes and were then analysed using UltraViolet (UV)-Double Beam Spectrophotometer (Systronics India Limited, Ahmedabad, India)(Figure 8) at 550 nm wave length with 65% concentrated nitric acid as the blank and the readings were recorded as Absorbance Units(AU). The absorbance values were indicative of the sealing ability of the tested furcation repair materials.

Figure 3: Furcal perforation

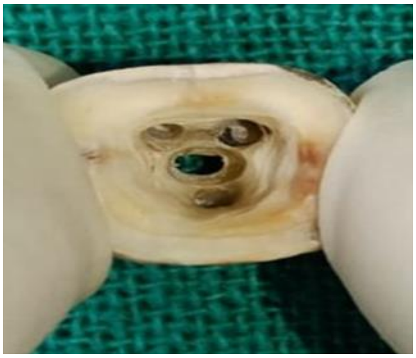


Figure 4: Furcal perforation sealed with repair material and root canal orifices sealed with sticky wax

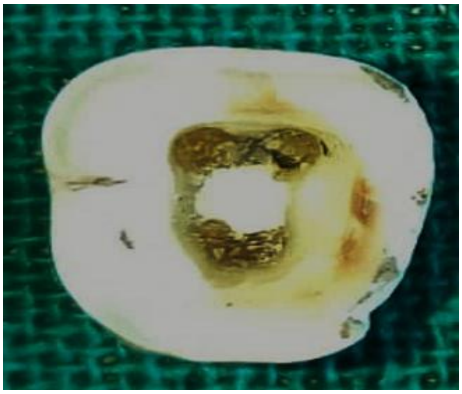


Figure 5: MTA REPAIR HP

Figure 6: BIODENTINE

Figure 7: WHITE MTA

Figure 8: Ultraviolet Double Beam Spectrophotometer

Results

The recorded readings of the absorbance units of all specimens were tabulated and statistically analysed with computer software; Statistical Package for Social Sciences(SPSS) version 24, Using Analysis of variance(One Way ANOVA) and Tukey's - Post hoc test. Analysis of Variance tests the equality of three or more means at one time by using variances.One way ANOVA showedstatistically significant difference in the mean values of the absorbance units between the groups as $P \leq 0.05$ (Table 1).It was found that Group A; in which MTA Repair HP was used as furcation repair material showed the best or maximum sealing ability with the Mean and Standard Deviation(SD)absorbance values of 0.206±0.005compared to Group B; Biodentine with 0.211±0.006& Group C; WMTA with 0.288±0.003 used as furcation repair materials and White MTA showed poor or least sealing ability (Table 1).

Table 1: Analysis of Variance (One Way ANOVA)

GROUPS	Number of specimens	P value	Absorbance Units (Mean ± SD)
Group A (MTA Repair HP)	20	≤ 0.05	0.206±0.005
Group B (Biodentine)	20		0.211±0.006
Group C (WMTA)	20		0.288±0.003
Negative control	10		0.056±0.001
Positive control	10		0.608±0.001

*P: Probability, *SD: Standard Deviation

To find where exactly the statistically significant difference is, Tukey's-Post hoc test was done for inter-group comparison between the five groups (Table 2). There was no statistically significant difference ($P > 0.05$) in the sealing ability of MTA Repair HP (Group A) and Biodentine (Group B) as furcation repair materials. However, Statistically significant difference ($P \leq 0.05$) was seen in the sealing ability between MTA Repair HP (Group A) and White MTA (Group C), Biodentine (Group B) and White MTA as furcation repair materials.

Table 2: Tukey's-Post hoc test was done for inter-group comparison between the five groups

Inter-Group Comparison	Comparative difference in the Mean values	Tuckey's Post hoc test P-value
Group A Vs Group B	-0.04	P=0.07 (NS)
Group A Vs Group C	-0.082	P<0.01
Group A Vs Negative control	0.150	P<0.01
Group A Vs Positive control	-0.402	P <0.01
Group B Vs Group C	-0.078	P<0.01
Group B Vs Negative control	0.154	P<0.01
Group B Vs Positive control	-0.398	P<0.01
Group C Vs Negative control	0.232	P<0.01
Group C Vs Positive control	-0.320	P<0.01
Negative control Vs Positive control	-0.552	P<0.01

*P: Probability, *NS: Non-Significant

Figure 9: Horizontal bar graph of Group A (MTA Repair HP) – Dye leakage in Absorbance Units

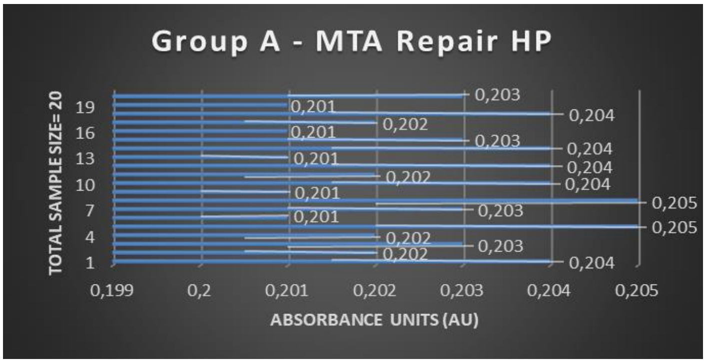


Figure 10: Horizontal bar graph of Group B (BIODENTINE) – Dye leakage in Absorbance Units

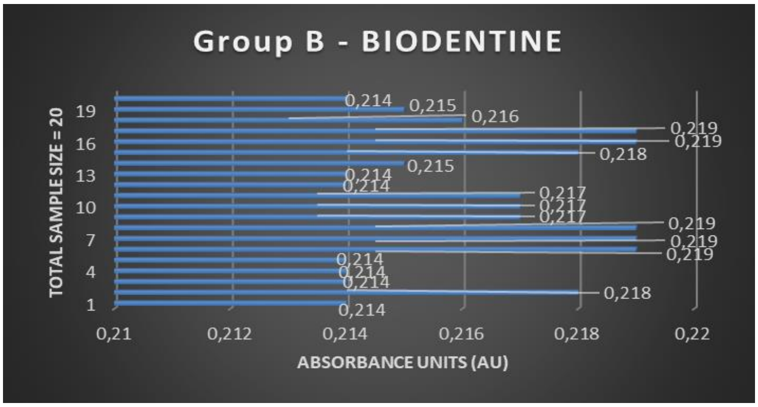
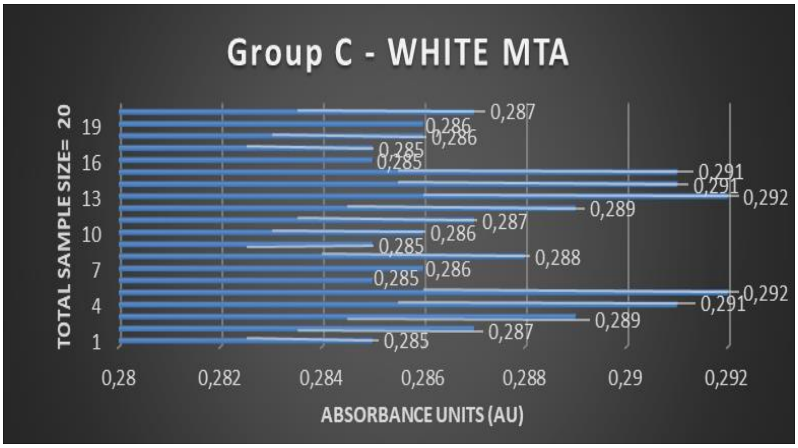


Figure 11: Horizontal bar graph of Group C (WHITE MTA) – Dye leakage in Absorbance Units



Discussion

This study found that MTA Repair HP cement showed maximum sealing ability as furcation repair material compared to Biodentine and White MTA (Figure 9; Figure 10; Figure 11). The findings of our study is not in accordance with the previous studies of(Grover*et al.,* 2020; Silva *et al.,* 2016; Shivakumar et al., 2021)which stated that MTA Repair HP cement showed poor sealing ability and higher microleakage as furcation repair material compared to biodentine. However, MTA Repair HP cement is a novel formulation maintaining all the chemical and biological characteristics of the original MTA. In MTA Repair HP, bismuth oxide is replaced with calcium tungstate as a radiopacifier(Silva *et al.*, 2016)and itmaintains the colour stability and does not result in tissue discolouration owing to lack of bismuth oxide. Calcium tungstate also contributes to higher calcium release, promoting higher biomineralization, antibacterial and optimal biological properties(Duarte*et al.,* 2012; Huffman *et al.,* 2009).The addition of plasticizer included in the liquid formulation of this cement favoured the reduction of its setting time and the short setting time is also correlated with the precursor powder higher surface area, the absence of compositional sulphate phases and its high aluminum content. Calcium sulphate affects the rate of chemical bonding, as lesser the sulphate, shorter the setting time of the cement(Jimenez-Sanchez *et al.,* 2019).The tricalcium silicate particles in MTA Repair HP ensures a very close contact between the calcium silicate and calcium aluminate, thus favouring the hydration reaction and the hydrated calcium aluminate silicate formed is responsible for its high biocompatibility, speed of the setting reaction, colour stability and can be used as endodontic repair cement(Jimenez-Sanchez *et al.,* 2019;Jimenez-Sanchez *et al.,*2020).The cement also showed better bond strength, resistance against thrust and is more durable to occlusal stresses (Aguiar*et al.,* 2019).Commonly used root canal irrigants like sodium hypochlorite, chlorhexidine gluconate and EDTA[Ethylene DiamineTetraacetic Acid] solutions do not cause darkening of MTA Repair HP cement(Metlerska*et al.*, 2021).

However this study also found that there was no significant difference in the sealing ability between MTA Repair HP and Biodentine. In a previous study of (Han *et al.,* 2011) showed the presence of calcium and silicon ions uptake into the dentin leading to the formation of tag-like structures in biodentine and the better sealing ability with biodentine can also be attributed to its modified powder composition, smaller particle size and liquid contains calcium chloride as setting accelerator and the addition of hydrosoluble polymer systems described as water reducing agents or super plasticizers, helps in maintaining the balance between the low water content and consistency of the mixture(Kadhim*et al.,* 2017).

Our study also showed biodentine is superior in its sealing ability as furcation repair material compared to WMTA and is in accordance with the previous studies of (Hassan *et al.,* 2015; Kumar *et al.,* 2016). The disadvantages of WMTA are its long setting time of 4 hours, difficulty in handling of the material and as it is prepared by mixing its powder with sterile water in a ratio of 3:1 ratio, any difference in this ratio will compromise its properties leading to its poor sealing ability(Hassan *et al.,* 2015).

In a previous study by (Wu *et al.,* 1998) stated that the calcium oxide contained in WMTA reacts with water and forms calcium hydroxide that discolours methylene blue dye. So in our study we used Indian ink dye, as it don't get discoloured with WMTA. In a previous study by (Oztan*et al.,* 2001)also reported Indian ink dye as a more reliable and suitable tracer in dye leakage studies than methylene blue dye. The linear dye penetration technique was the most commonly used method to evaluate the sealing ability of any dental cements, because of its ease of performance and difficulty of other available techniques. Despite its popularity, it has several drawbacks – as it relies randomly on sectioning of tooth into two pieces, without any clue of the site of deepest dye penetration in the sectioned tooth specimens, the depth of dye penetration is not uniform around the margins of restorations and gives randomly chosen results, raising doubts about their reliability, doesn't measure the actual volume of the dye absorbed by the specimens, but merely measures the deepest point reached by the dye (Camps *et al.,* 2003).This drawback is avoided in Dye extraction method, as here the teeth are completely dissolved in concentrated acids, thus releasing the absorbed dye from the interface and it takes into account all the absorbed dye by the specimens, after which the optical density of the obtained solution is measured by Ultraviolet-Spectrophotometer(Sikri., 2010).Our study followed an established protocol for dye extraction method.

According to the Beer-Lamberts law, the absorbency of the solution is directly proportional to the concentration of absorbing species in the solution and path length. Thus for a fixed path length, Ultraviolet-Spectroscopy can be used to determine the amount of the absorber (Indian ink dye) in the solution and hence it can be interpreted that absorbance of the solution is directly related to the amount of the dye leaked along the tooth-cement interface and is recorded as absorbance units(Naik e*t al.,* 2015). So in our study, we evaluated the sealing ability of furcation repair materials using Ultraviolet-Spectrophotometer analysis.
In our study, Negative control group specimens showed low dye absorbance values with Mean and SD of 0.056 ± 0.001 (Table 1) close to that of blank nitric acid which showed absorbance value of 0.030 and this small difference was attributed to the yellowish colour of teeth, where as blank nitric acid is colourless. In Positive control group specimens, perforations were not repaired and they showed the highest dye absorbance valueswith Mean and SD of 0.608 ± 0.001 (Table 1) compared to all the other group specimens denoting the accuracy of the technique used.

Conclusion

Within the limitations of this study, it was found that MTA Repair HP showed better sealing ability compared to Biodentine and WMTA as furcation repair material. Therefore, MTA Repair HP endodontic restorative cement can be used as replacement for Biodentine and WMTA as furcal repair material. However, further in-vivo studies are recommended to confirm and correlate the findings of this in-vitro study to a clinical scenario.

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