



Evaluation of the sealing ability of White Mineral Trioxide Aggregate, Biodentine and Mineral Trioxide Aggregate Repair HP as root end filling materials with two different Retro-cavity preparation techniques. An in-vitro Ultraviolet-Spectrophotometric analysis.

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Abstract:

This study aims to evaluate sealing ability of White Mineral Trioxide Aggregate, Biodentine and Mineral Trioxide Aggregate Repair HP as root end filling materials with two different Retro-cavity preparation techniques by in-vitro Ultraviolet-Spectrophotometric analysis. 140 extracted human mandibular single rooted premolar teeth were included. In all specimens, Endodontic access cavity preparations were done, pulp tissue was extirpated and working length was established. Biomechanical preparation was done in step-back technique with K-file no.40 as master apical file along with the use of root canal irrigants. Root canals were then dried, coated with AH-plus sealer, obturated with guttapercha by cold lateral condensation technique and access cavities were restored with Glass ionomer cement. In all the specimens, the apical 3mm of roots were resected and were then divided into 2 groups; Experimental group with 120 specimens and Control group with 20 specimens. Experimental group is divided into Group-I and Group-II with 60 specimens each. 3mm depth of retro-cavity preparations were done. In Group-I, Class-1 Retro-cavity preparation was done using diamond bur and in Group-II, Retro-cavity preparation was done using Pro-ultrasonic tip. Group-I and II were further divided into three groups each with 20 specimens per group. In Group-IA and IIA; White MTA, Group-IB and IIB; Biodentine, Group-IC and IIC; MTA Repair HP were used as root end filling materials. All specimens were immersed in Indian ink dye for 48 hours and were then subjected to dye extraction method, 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. Retro-cavity preparation using ultrasonic tip with Biodentine as root end filling material showed the maximum sealing ability. However, there was no significant difference in the sealing ability between Biodentine and MTA Repair HP as root end filling material.

Key words: Biodentine; MTA Repair HP; Root end filling; Sealing ability; White MTA.

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Introduction:

The goal of root canal treatment is cleaning, shaping and complete obturation of root canal system, thus preventing the proliferation of microorganisms and their by-products. When root canal treatment fails, Non-surgical retreatment is the common treatment of choice.¹ According to Bergenholtz et al.,² Non-surgical retreatment usually results in successful outcome. However, when a non-surgical root canal treatment proves unsuccessful because of the complexity of root canal system, inadequate instrumentation, presence of post-core restorations and separated instruments in the apical-thirds of root canals, in such cases surgical endodontic therapy becomes the only alternative to save the involved tooth.

Surgical endodontic therapy involves the elevation of mucoperiosteal flap, osteotomy, location of root tip, root end resection, preparation of root end cavity or retro-cavity and restoring with root end filling material. 98% of ramifications and 93% lateral canals are seen in the apical one-third of the roots, so root end resection becomes indispensable in surgical endodontics.³ The depth of root end cavity preparation should be ideally 3mm and more than this depth does not bestow any greater benefits, whereas lesser depth jeopardizes the long-term success of the apical seal.⁴ Root end filling is the procedure by which an inert, biocompatible material is packed into the

root end cavity. Gartner AH et al.,⁵ explained the relation between the success of surgical endodontic therapy and properties of ideal root end filling materials. An ideal root end filling material should adhere to the tooth tissue and seal the root end three-dimensionally forming a tight seal in the root canal system. It should be non-toxic, well tolerated by periradicular tissues, promote healing and regeneration of periradicular tissues, radiopaque, easy to manipulate, sets rapidly, dimensionally stable, non-resorbable, have bactericidal or bacteriostatic properties and not be affected by presence of moisture or low pH levels.⁶

The most preferred root end filling material in endodontics are bioactive materials like Mineral Trioxide Aggregate (MTA) and Biodentine. MTA is basically available as Gray MTA and White MTA (WMTA). MTA is mainly composed of calcium oxide, dicalcium silicate, tricalcium silicate, tricalcium aluminate, tetracalcium aluminoferrite and calcium sulphate dihydrate. However, White MTA lacks tetracalcium aluminoferrite. Gray MTA causes discolouration to tooth and gingiva. The absence of Iron oxide in White MTA causes the colour change from Gray to White.⁷ The initial setting time of WMTA is approximately 4 hours. During the initial stages of its setting, the pH is 10.2 and upon its final setting it increases to 12.5. WMTA 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.⁸Biodentine 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 by forming tag-like structures at its interface with the tooth structure.⁹Recently, a new formulation of MTA was introduced; Mineral Trioxide Aggregate (MTA) Repair HP, is an endodontic restorative cement with HighPlasticity(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.¹⁰

Apart from the root end filling materials, the success of surgical endodontic therapy also depends on the retro-cavity preparation technique used. Retro-cavity preparation using conventional burs had many disadvantages and were overcome with the use of ultrasonic tips which helped in creating a more conservative, smooth, more centrally placed Retro-cavities, thus decreasing the number of exposed dentinal tubules at the resected root surface thereby reducing apical leakage.³

There is not much data to determine the sealing ability of MTA Repair HP as root end filling material in endodontics. So, this study aims to evaluate the sealing ability of White MTA, Biodentine and MTA Repair HP as root end filling materials with two different Retro-cavity preparation

techniques using Ultraviolet-Spectrophotometric analysis.

Materials and Methods:

One hundred forty freshly extracted human permanent mandibular single rooted premolar 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, single rooted and single canal teeth. Exclusion criteria: Carious, fractured, restored, open root apices, multi-rooted and multiple canal teeth. 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.

Standardized endodontic access cavity preparations were done in all the specimens using Endo access bur No. 2 (Dentsply, Maillefer, Switzerland) in a high speed contra-angled handpiece (NSK, Japan), pulp tissue was extirpated using barb broaches (Mani, Japan). K(Kerr)-file No.10 (Mani, Japan) was placed into the root canal to establish the patency till the apical foramen and the working length was determined by subtracting 0.5mm from the length achieved with the tip of the trial file just visible at the apical foramen of each root canal in all the specimens. Biomechanical preparation was done in step-back technique using K-file ISO(International Organization for Standardization) No.15 upto K-file ISO No.40(Mani, Japan) as master apical file and during the biomechanical preparation, 17% EDTA (EthylineDiamineTetraAcetic acid) (Prime Dental Product Pvt, Ltd,



Thane, India) and 3% Sodium hypochlorite (Neelkaanth Health Care Pvt Ltd, Ahmedabad, India) were used as root canal irrigants. The root canals were then dried with sterile paper points (Dia Dent International, Korea) and were then coated with a thin mix of AH(Amine Hydroxy) - Plus root canal sealer (Dentsply Maillefer, DeTray, Germany).Guttapercha point (2% Taper, Dia Dent International, Korea) ISO No.40 size was used as master apical cone and it was lightly coated with AH-Plus sealer and slowly inserted into the root canal until it reaches the full working length. Accessory guttapercha points of ISO No.30,20,15 sizes were also coated with AH-Plus sealer, placed into the root canals and were obturated by cold lateral condensation technique until these accessory guttapercha points could not be cold laterally condensed more than 5mm into the root canal space. A heated finger Plugger (Mani, Japan) was used to remove the excess guttapercha to a level 2mm short of the canal orifice and the guttapercha was then vertically condensed with a cold finger plugger. The quality and apical extent of root canal obturations were assessed with digital radiographs (Radiovisiography, Carestream Dental, Kodak 5200, France) in bucco-lingual, mesio-distal directions and root canal obturations were deemed satisfactory with the presence of uniform radioopacity reaching upto the pre-determined working length in all specimens. The access cavities in all the teeth were then restored with Light-cure Glass ionomer cement (GC Corporation, Tokyo, Japan). All the specimens were then stored at 37⁰C in 100% relative humidity for five days to allow the root canal sealer to completely set. In all the specimens, the apical 3mm of roots were resected perpendicular to its long axis (90-degree angle) with a straight

fissure bur (Mani, Japan) in a high speed contra-angled handpiece along with water coolant.

All the 140 specimens were then divided into 2 groups; Experimental group with 120 specimens and Control group with 20 specimens. Experimental group is divided into Group-I and Group-II with 60 specimens each. A 3mm depth of retro-cavity preparations were done. In Group-I, Retro-cavity preparation was done using standard no. BC-32 round diamond bur (Mani, Japan) in a high speed contra-angled handpiece (Figure 1). In Group-II, Retro-cavity preparation was done using Pro-ultrasonic tip No.2 (Dentsply Maillefer, Dentsply India Pvt. Ltd, Mumbai) at low intensity of 4MHz following manufacturer's instructions (Figure 2; Figure 3). Group-I was further divided into three groups with 20 specimens per group. In Group-IA; White MTA, Group-IB; Biodentine, Group-IC; MTA Repair HP were used as root end filling materials. Group-II was also further divided into three groups with 20 specimens per group. In Group-IIA; White MTA, Group-IIB; Biodentine, Group-IIC; MTA Repair HP were used as root end filling materials. Control group was divided into positive control and negative control with 10 specimens each. In positive control specimens, retro-cavity preparations were done using round diamond point and were not restored with any root end filling materials. In negative control specimens, neither retro-cavity preparations were made nor restored with any root end filling materials.

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 retro-cavities in Group; IA and IIA specimens using a messing carrier (AMT Dental Pvt. Ltd. Navi Mumbai) and gently condensed with finger pluggers upto 3mm thickness (Figure 4).

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 retro-cavities in Group; IB and IIB using an amalgam carrier (GDC, Mumbai) and gently condensed with finger pluggers upto 3mm thickness (Figure 5).

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 retro-cavities in Group; IC and IIC using a messing carrier and gently condensed with finger pluggers upto 3mm thickness (Figure 6).

All the specimens were then stored at 37⁰C in 100% relative humidity for 48hours to allow the root end filling materials to completely set and were then subjected to thermocycling at 4⁰±2⁰C and 56⁰±2⁰C for 500, 2minute cycles with a dwell time of 30 seconds as per International Organization for Standardization Standard 11405.¹¹ All the experimental and positive control group specimens were double coated with nail varnish except in the apical 3mm of roots. Whereas in negative control group specimens, the entire tooth

was double coated with nail varnish. 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, ThermoScientific 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 7) at 550 nm wave length with 65% concentrated nitric acid as the blank and the readings were recorded for each specimen as Absorbance Units(AU). The absorbance values were indicative of the sealing ability of root end filling materials and retro-cavity preparation techniques used. As lower the absorbance units; better the sealing ability and higher the absorbance units; poor the sealing ability with either root end filling material or retro-cavity preparation techniques used in this study.



Figure 1: Retro cavity preparation with round bur



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Figure 2: Pro-ultrasonic tip no. 2



Figure 3: Retro-cavity preparation with Pro-ultrasonic tip no. 2



Figure 4: WHITE MTA



Figure 5: BIODENTINE



Figure 6: MTA REPAIR HP



Group IIA	20	<0.05	0.39 ± 0.001
Group IIB	20		0.23 ± 0.022
Group IIC	20		0.24 ± 0.019
Positive control	10		0.4 ± 0.001
Negative control	10		0.06 ± 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 in retro-cavity preparation techniques. Statistically significant difference(P value is < 0.05) was seen between the Group; IB and IIB, Group; IC and IIC, indicating that

retro-cavity preparation technique with ultrasonic tip showed better sealing ability of the root end filling materials to the prepared walls of root end cavities compared to the retro-cavity preparation technique with round bur (Table 2) (Figure 9; Figure 10; Figure 13).

Table 2: Tukey’s post-hoc test for Inter-group comparison

Inter-group Comparison	Comparative difference in the Mean values	Tukey’s post-hoc test P value
Group IA Vs Group IIA	0.04	<0.01
Group IB Vs Group IIB	0.02	0.04
Group IC Vs Group IIC	0.02	0.04
Group IA Vs Positive control	0.03	0.001
Group IB Vs Positive control	-0.15	<0.01
Group IC Vs Positive control	-0.14	<0.01
Group IA Vs Negative control	-0.37	<0.01
Group IB Vs Negative control	-0.19	<0.01
Group IC Vs Negative control	-0.20	<0.01
Group IIA Vs Positive control	-0.01	<0.01
Group IIB Vs Positive control	-0.17	<0.01



Group IIC Vs Positive control	-0.16	<0.01
Group IIA Vs Negative control	0.33	0.001
Group IIB Vs Negative control	0.17	0.001
Group IICVs Negative control	0.18	0.001

***P:** Probability

However, to find where exactly their was no statistically significant difference seen, Tukey’s post-hoc test was done for intra-group comparison of the three root end filling materials used and it was found that their was no statistically significant difference (P value is> 0.05) in the sealing ability of Biodentine and MTA Repair HP (Between Group; IB and IC, Group; IIB and IIC) as root end filling materials (Table 3).

Table 3: Tukey’s post-hoc test for Intra-group comparison

Intra-group Comparison	Comparative difference in the Mean values	Tukey’s post-hoc test P value
Group IA Vs IB	0.18	0.001
Group IB Vs IC	-0.01	0.85 NS*
Group IA Vs IC	0.17	0.001
Group IIA Vs IIB	0.16	0.001
Group IIB Vs IIC	-0.01	0.68 NS*
Group IIA Vs IIC	0.15	0.001

***P:** Probability, ***NS:** Non-Significant

Figure 8: Horizontal bar graph of Group IA (Retro-cavity preparation with Bur + WMTA as Root end filling material) – Dye leakage in Absorbance Units



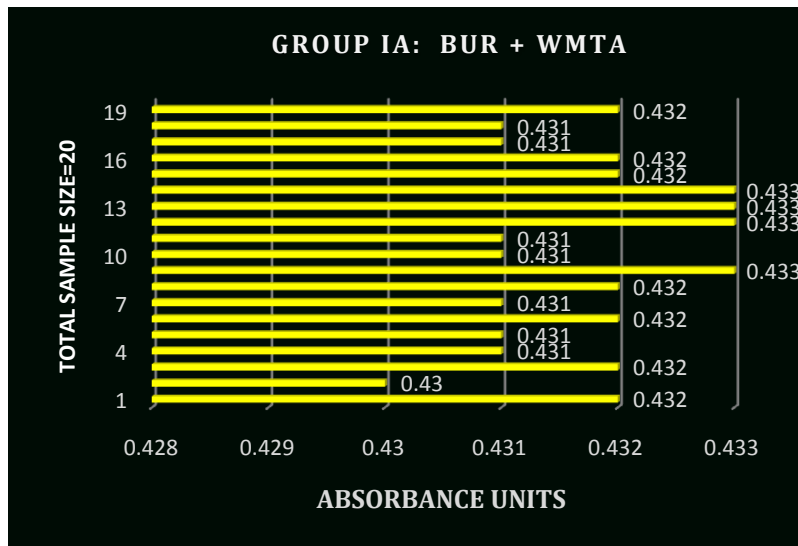


Figure 9: Horizontal bar graph of Group IB (Retro-cavity preparation with Bur + Biodentine as Root end filling material) – Dye leakage in Absorbance Units

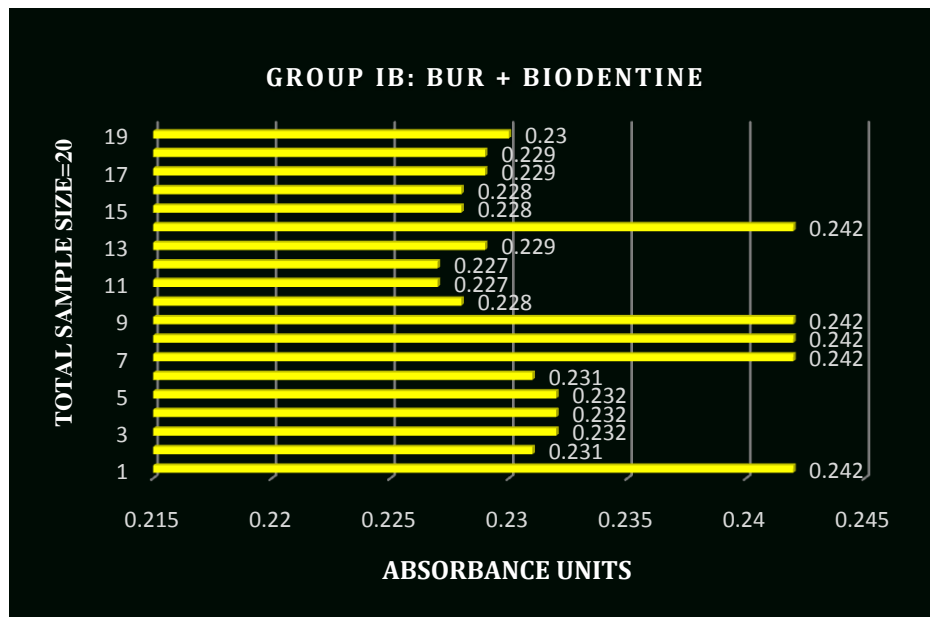


Figure 10: Horizontal bar graph of Group IC (Retro-cavity preparation with Bur + MTA Repair HP as Root end filling material) – Dye leakage in Absorbance Units

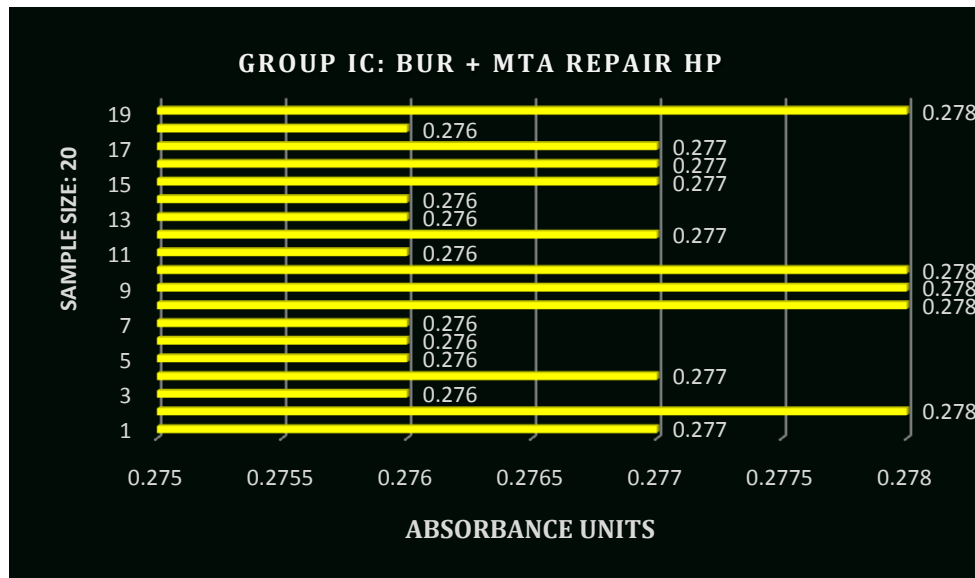


Figure 11: Horizontal bar graph of Group IIA (Retro-cavity preparation with ProUltrasonic tip + WMTA as Root end filling material) – Dye leakage in Absorbance Units

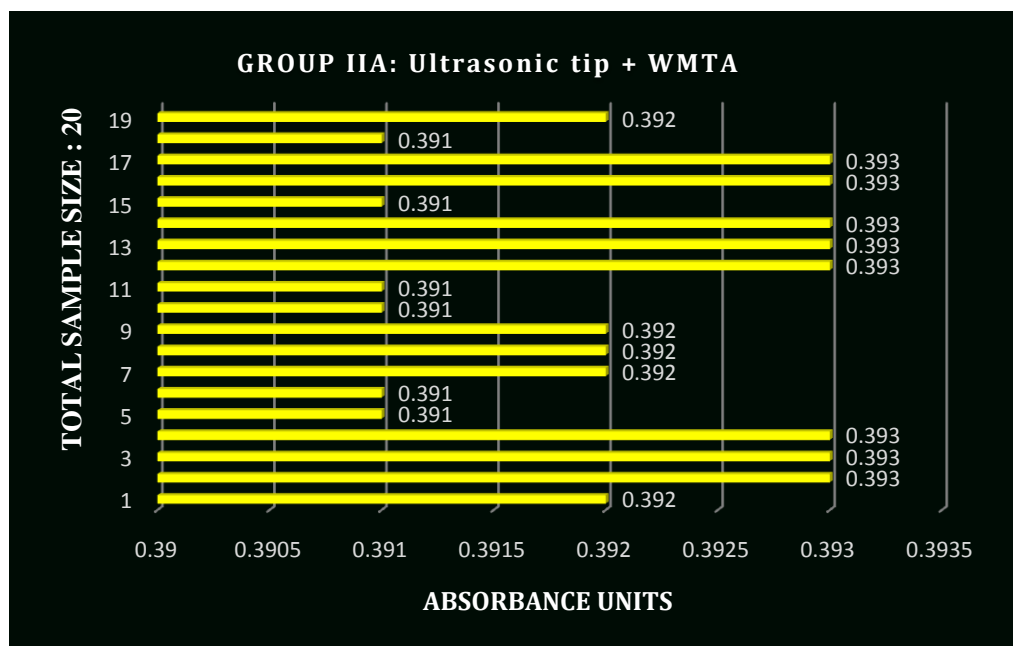


Figure 12: Horizontal bar graph of Group IIB (Retro-cavity preparation with ProUltrasonic tip + Biodentine as Root end filling material) – Dye leakage in Absorbance Units



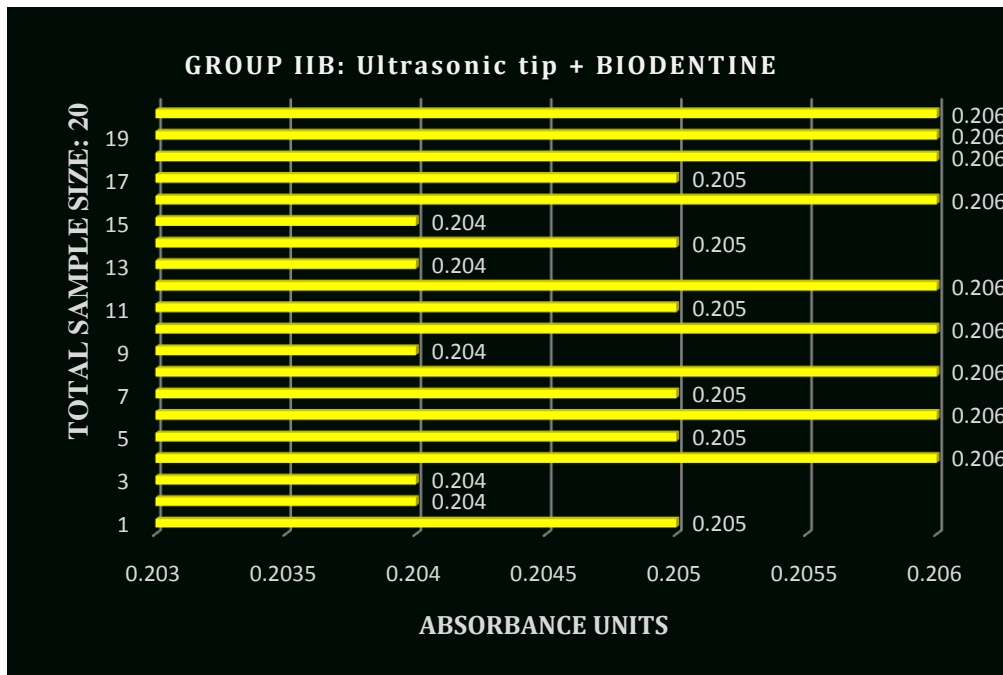
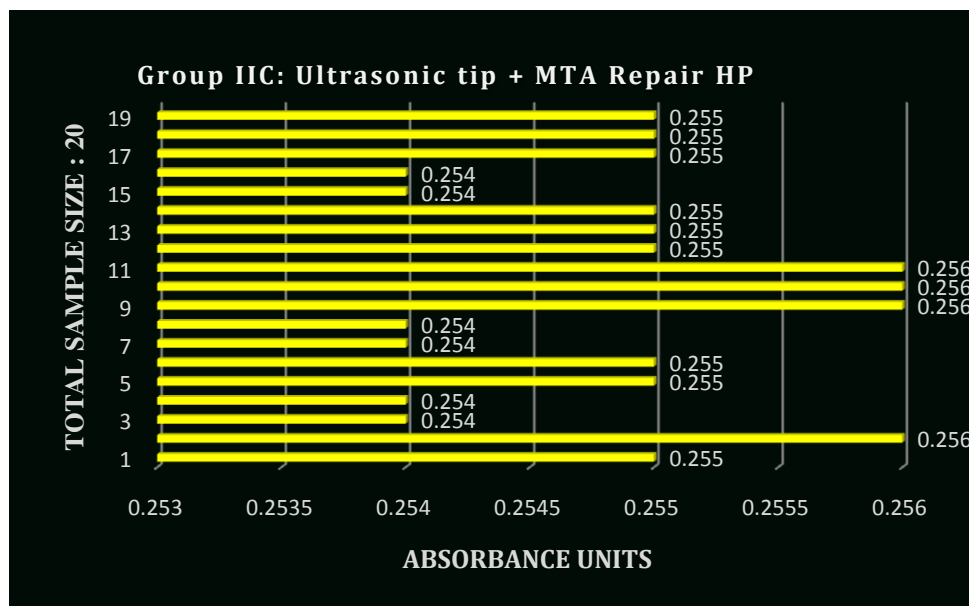


Figure 13: Horizontal bar graph of Group IIC (Retro-cavity preparation with ProUltrasonic tip + MTA Repair HP as Root end filling material) – Dye leakage in Absorbance Units



Discussion:

Success of root canal treatment depends upon complete disinfection of root canal space by thorough cleaning and shaping followed by 3-dimensional obturation in order to achieve a fluid impervious seal of the root canal system and when this fluid tight seal cannot be obtained by orthograde

root canal treatment, surgical endodontic therapy of the tooth is needed.¹²Surgical endodontic treatment comprises of through debridement of pathological periradicular tissues, root end resection followed by retro-cavity or root end cavity preparation and placement of root end filling material into the prepared cavity in order to



completely seal the root end against microleakage.¹³

With the recent development of ultrasonic retro-tips, major shortcomings of using rotary burs in the retro-cavity preparation of the resected roots were overcome. Root end cavity preparation with ultrasonic tips helps in preparing more conservative cavities compared to the conventional burs.¹⁴ In our study, retro-cavity or root end cavity preparation with ultrasonic tip provided better sealing ability of the root end filling materials used compared to the use of conventional round burs and this is in accordance with the previous studies reporting that ultrasonic retro tips showed cleaner, well-centered cavities and are free of smear layer, thus helping for the good marginal adaptation of root end filling materials to the tooth structure preventing microleakage. Whereas the root end cavities prepared with the conventional burs resulted in the formation of considerable amount of debris, smear layer and these remnants are permeable to fluids and toxins thus preventing the intimate contact of the root end filling materials to the prepared retro-cavity walls.^{15,16,17}

The disadvantages of WMTA are its difficulty in handling of the material, as it is prepared by mixing its powder with sterile water in a ratio of 3:1 ratio and any small difference in this ratio will compromise its properties leading to its poor sealing ability.¹⁸ MTA Repair HP cement exhibits all the chemical and biological characteristics of the original Pro-root MTA. Here bismuth oxide is replaced with calcium tungstate as a radiopacifier and it maintains 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.^{19,20} 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's 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.²¹ 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, the hydrated calcium-aluminate-silicate formed is responsible for its optimal biocompatibility, speed of the setting reaction, colour stability and can be used as endodontic repair cement.^{21,22}

In our study, Biodentine showed the maximum sealing ability compared to WMTA and MTA Repair HP as the root end filling material, irrespective of the retro-cavity preparation techniques used. In a previous study, Han et al⁹ stated that biodentine showed the presence of calcium and silicon ions uptake into the dentin leading to the formation of tag-like structures alongside an interfacial layer called the "mineral infiltration zone," where the alkaline caustic effect of calcium silicate cement hydration products degrades the collagenous component of interfacial dentin. 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.^{23,24}

The present study is also in accordance with the previous studies of Shivakumar et al,²⁵ Shivakumar HS et al²⁶ and Yogesh K et al²⁷ reported that Biodentine has the lowest dye penetration, microleakage and better sealing ability compared to MTA Repair HP and WMTA. Biodentine has better handling and mechanical properties than MTA Repair HP and the faster setting time seals the interface, reducing the risk of bacterial contamination.²⁵ Biodentine particles are smaller in size and the porosity of the sealing surface, the pore volume in the set material are lower than in WMTA, explaining the reason for its better sealing ability.²⁸

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.²⁹ 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.³⁰ Our study followed an established protocol for dye extraction method.

Ultraviolet-Spectrophotometric analysis determines the amount of the absorber (Indian ink dye) in the solution and is 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.³¹ So in our study, we evaluated the sealing ability of three root end filling materials with two different Retro-cavity preparation techniques using Ultraviolet-Spectrophotometer analysis.

Conclusion:

Within the limitations of this study it was found that; 1. Retro-cavity preparation with ultrasonic tip followed by the placement of root end filling materials showed better sealing ability than the use of conventional burs for retro-cavity preparation. 2. Irrespective of the retro-cavity preparation technique used, Biodentine as root end filling material showed better sealing ability than MTA Repair HP and WMTA. However, there was no significant difference in the sealing ability between Biodentine and MTA Repair HP. 3. Retro-cavity preparation using ultrasonic tip with biodentine as root end filling material showed the maximum sealing ability. 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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