ORIGINAL RESEARCH

Efficacy of Mineral Trioxide Aggregate Repair High Plasticity, Biodentine, and EndoSequence Root Repair Material Putty as Apical Barriers in Immature Permanent Teeth: An *In Vitro* Bacterial Leakage Study

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Abstract

Aim: To assess the microleakage of mineral trioxide aggregate (MTA) repair high plasticity (HP), EndoSequence root repair material (ESRRM) putty, and Biodentine, when used as an apical plug in immature permanent teeth.

Materials and methods: In an *in vitro* model, 55 extracted maxillary incisors were decoronated and resected 3 mm apically to obtain standardized 15-mm root blocks, which were then cleaned and shaped. All samples had a 1.1-mm standardized, prepared artificial open apex. The teeth were arbitrarily designated into three experimental groups ($n = 15$) and two control (positive/negative) groups ($n = 5$). In the experimental groups, orthograde 4-mm thick apical plugs of Biodentine (group I), ESRRM putty (group II), and MTA repair HP (group III) were placed. Positive control samples were left vacant while negative control samples were filled with Biodentine. The bacterial leakage method was used to appraise the sealing efficiency of the cements.

Results: Statistical package for the social sciences (SPSS) software, version 21.0, was used for data analysis. *Post hoc* Tukey's test, one-way analysis of variance (ANOVA), and repeated measures of ANOVA were used for intergroup and intragroup comparisons. On day 1, there was a significant difference between the groups, with group II showing the least and group 1 showing the maximum microleakage. No significant difference among the groups was seen at other observational periods. There was a tendency for leakage to increase significantly from day 1 to 7, then decrease till the end of the experimental period.

Conclusion: It was concluded that the three materials evaluated, with time, exhibited comparable apical microleakage when treating teeth with open apices.

Clinical significance: MTA repair HP can be used as an apical plug material in open apices with similar success as ESRRM putty and marginally better outcome than Biodentine.

Keywords: Apical plug, Bacterial leakage, Biodentine, EndoSequence root repair material putty, mineral trioxide aggregate repair high plasticity.

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Introduction

Adolescents and children are commonly afflicted with dental trauma, especially involving the anterior teeth, the prevalence of which can range from 6.1% to 58.6%.[1]

This trauma may lead to pulpal necrosis of a young permanent tooth which can further cease the complete development and maturation of the root dentin and closure at the apex. This deficiency in root formation when treated with traditional endodontics usually fails. This could be due to root fracture of the friable root dentin or the inability to thoroughly clean and obturate the root due to lack of an apical stop.[2,3]

Apexification procedures are the standard treatment for such immature teeth.[3] In apexification with multiple visits, calcium hydroxide is still the most widely used material. Even though it develops a physiological solid tissue barrier, it possesses certain fallacies such as prolonged treatment time, increased root fracture incidence, coronal microleakage, poor sealing capacity, and setting problems.[3] All of the aforementioned issues can be acknowledged with bioactive endodontic cements (BECs), like MTA.[4,5]

Mineral trioxide aggregate is considered the gold standard and has been proven to be biocompatible, with excellent sealing capacity, which has made single-visit apexification, a progressively attractive procedure.[6] However, it has some clinical drawbacks, including poor handling properties and an extended setting time.[7] As a result, to avoid this, researchers

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and manufacturers have developed new BEC formulations and products that do not compromise MTA's bioactivity or biocompatibility.[8]

Biodentine, a calcium silicate-based material, has notable clinical characteristics, namely, enhanced sealing ability, increased compressive strength, increased density and decreased porosity, optimum bioactivity, enhanced remineralization capability, and color stability in comparison to MTA.[2,6] As Biodentine has similar endodontic implications to MTA, its use as a root-end filling material can be explored.[2]

EndoSequence Root Repair Material is a premixed bioceramic material that comes in an injectable paste or putty form that is said to be convenient to physically handle and place where needed.[9] As a retrograde restorative material, it has shown comparable sealing ability as that of Biodentine.[10]

Mineral trioxide aggregate repair HP, a novel formulation of white MTA with newer radiopacifier such as calcium tungstate ($CaWO_4$), was used to improve the material's biomechanical and physicochemical properties. This product is altered to a liquid state by adding a biological plasticizer to distilled water, which increases plasticity and thus improves manipulation.[5,11,12]

A material must exhibit an excellent seal apically to accomplish a favorable outcome after an apexification procedure.[13] There is limited research on apical microleakage evaluation of Biodentine and ESRRM putty when used as an orthograde restorative material.[7,10] The studies state that both materials show comparable results.[7,10] As both materials are considered the gold standard among calcium silicate-based types of cement and with no literature that compares the sealing ability of MTA repair HP as an orthograde apical plug to that of Biodentine and ESRRM putty, this experiment intended to assess and compare the apical microleakage of Biodentine, ESRRM putty, and MTA repair HP in an open apex model using a bacterial leakage method.

Materials and Methods

Study Design and Sample Size

This experimental *in vitro* study (institutional ethical approval No.: KIMS/KIIT/IEC/98/2016) was carried out from 1 September 2019 to 31 March 2020. This study was a single-blinded study, where the person involved in outcome assessment was blinded to avoid detection bias. Sample size estimation was done using G-power software, version 3.0. A total sample size of 55 (15 per group and 5 for controls) was found to be sufficient for an alpha of 0.05, 95% power, and 0.5 effect size. Thus, a total of 55 human maxillary incisors were chosen.

Inclusion Criteria

- Single-rooted teeth extracted for periodontal or orthodontic concerns.
- Teeth without root aberrations, resorption, or calcifications.
- Teeth without previous endodontic treatment, crowns, or posts.
- Teeth without any root fracture or craze lines.

Exclusion Criteria

- Multirooted teeth.
- Teeth with root fracture, craze lines, root aberrations, resorption, or calcifications.
- Teeth with previous endodontic treatment, crowns, or posts.

Tooth Preparation

Calculus and tissue remnants from selected teeth were removed with a scaler. The teeth were then autoclaved, cleaned with distilled water, and stored in normal saline until use.[2] A diamond disk was used to decoronate the teeth, leaving roots 18-mm in length. The root terminus was then amputated by 3 mm to remove apical ramifications and deltas. The remaining root length (15 mm) was negotiated with a No. 20 K File (Mani). The working length was visually determined, 0.5-mm deficit to the apex. Gates Glidden drills (Nos. 1, 2, and 3) were used for canal shaping of the coronal two-thirds, and hand files up to No. 40 K-File (Mani) were used for apical third canal shaping. To replicate the clinical scenario of a gaping apex, the apical ends of the roots were prepared retrogradely with Mani Peeso Reamers (Nos. 1, 2, and 3). Throughout the preparation, the canals were flushed with 5.25% NaOCl (2 mL) as each instrument was replaced. Canals were filled for 1 minute with 17% EDTA, followed by flushing with 5.25% NaOCl for 3 minutes, for smear layer removal. A 5 mL of normal saline was used for final irrigation.[4]

Experimental Design Setup and Grouping

The prepared roots were divided by a simple random sampling procedure using the Microsoft excel program into 3 test groups ($n = 15$), a positive and negative control group ($n = 5$). Before apical plug compaction, to emulate the soft tissues of the periapex, the root tips of the teeth were embedded in a moist sponge and the material was compacted.[4] Canals were dried using paper points before inserting incrementally, a 4-mm apical plug of root filling material in an orthograde direction. Each material was manipulated following the manufacturer's instructions. The following three groups were formed:

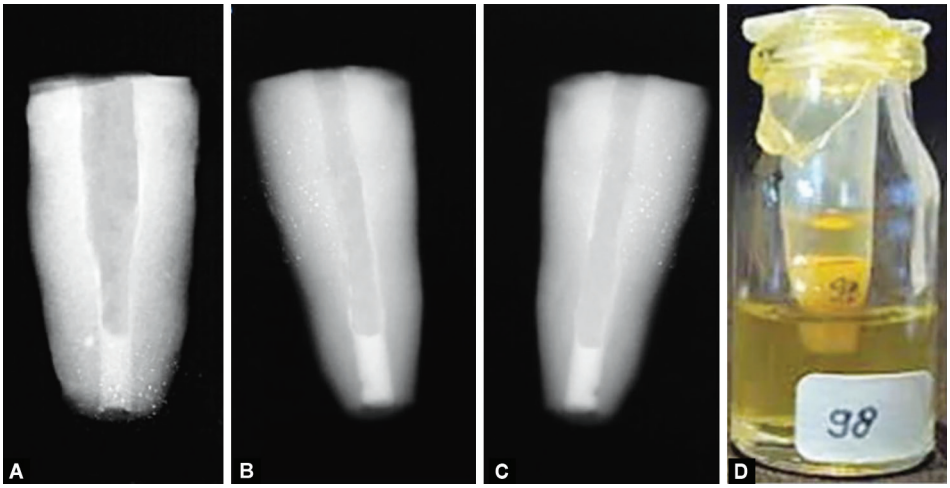
1. Group I, Biodentine (Septodont, France): The material was inserted in the canal using an MTA carrier and consolidated with pre-fitted pluggers.
2. Group II, ESRRP (Brasseler USA): The material was injected and condensed directly into the root canal.
3. Group III, MTA repair HP (Angelus, Brasil): The material was inserted in the canal using an MTA carrier and compressed with pre-fitted pluggers.

Radiographic verification of the apical plug's placement position was done (Figs 1A to C). The remaining canal space was left vacant. A temporary restorative material (Coltosol, Iran) was used to seal the canals. Root canals in the control groups were treated similarly to the test groups. The canals were left empty in the positive control, whereas, the canals were filled with Biodentine in the negative control samples.

All test samples were then incubated for 24 hours at 37°C and 100% humidity following which two layers of nail varnish were applied to the entire root, excluding the canal access and 2 mm from the apex of the test and positive control groups to impede seepage from any cemental tears or accessory canals into the main canal. For samples in the negative control group, the root surface in its entirety, with the apex and access was sealed with nail varnish and sticky wax.[4] After 24 hours the test samples were assessed for bacterial leakage.

Bacterial Leakage Test

The specimens were assembled on the bacterial leakage models (*Enterococcus faecalis*/ATCC strain 29212) after 24 hours, with 2–3 mm of the root tip immersed in the brain-heart infusion (BHI)



Figs 1A to D: Radiograph verification after placement of (A) Biodentine (group I); (B) ESRRP (group II); (C) MTA repair HP (group III); (D) Dual chamber bacterial leakage model setup

broth (Fig. 1D). The entire model was sterilized in an ultraviolet (UV) chamber. Furthermore, 9×10^8 CFU/mL of *E. faecalis* (ATCC 29212) was inoculated in 1 mL of BHI-broth, and the resultant suspension was used for top chamber replenishment every other day for 30 days. The turbidity of the media in the bottom chamber due to bacterial leakage was estimated using a UV spectrophotometer on days 1,7, 14, and 30. The resultant turbidity (optical density) was recorded as absorbance values which were used for microleakage estimation of the test samples. A sample from the culture tube was cultivated to affirm the pureness of *E. faecalis* in the BHI-broth.[2,4]

Statistical Analysis

The data was interpreted in SPSS software version 21.0 (IBM, Chicago, IL, USA). The inferential statistics comprised of one-way ANOVA for intergroup comparison and repeated measures of ANOVA along with *post hoc* Tukey's test for intragroup comparison. The significance level was kept at 0.05.

Results

In the observational timeline, all positive control samples exhibited high microleakage from day 1 itself, and samples in the negative control did not show any leakage. These results indicated that the test design was dependable and effective.

The mean absorbance values with standard deviation (SD) and one-way ANOVA for intergroup comparison across different timelines are summarized in Table 1. On intergroup comparison, group II (0.0004 ± 0.0012) showed the least microleakage followed by group III (0.0008 ± 0.0013) and group I (0.0015 ± 0.021) showed the maximum amount of microleakage on day 1, the difference of which was statistically significant ($F = 3.017$; $p = 0.033$). On day 7, group II (0.0024 ± 0.0036) showed the least microleakage followed by group III (0.0028 ± 0.0039) and group I (0.0035 ± 0.0047) showed the maximum amount of microleakage, although this difference was not statistically significant ($F = 0.984$; $p = 0.627$). On day 14, group II (0.0021±0.0028) showed the least microleakage followed by group III (0.0024 ± 0.0031) and group I (0.0025 ± 0.0106) showed the maximum amount of microleakage, although this difference was not statistically significant ($F = 2.567$; $p = 0.056$). On day 30, group II (0.0017 ± 0.0024) showed the least microleakage followed by group III (0.0018 ± 0.0022) and group I (0.0019 ± 0.0249) showed the maximum amount of microleakage, although this difference was not statistically significant ($F = 1.254$; $p = 0.892$).

On intragroup comparison of mean absorbance values (Table 2) using repeated measures of ANOVA and *post hoc* Tukey's test, it was found that the mean absorbance values of all three study groups increased significantly from day 1 to day 7 ($p < 0.001$), then decreased from day 7 to 14 and then from day 14 to 30, but this decrease from day 7 to 14 and from day 14 to 30 was not statistically significant.

The overall results indicate that group II showed the least microleakage followed by group III and I, the difference of which was significant only on day 1, but over the other timelines, it showed comparable results. The microleakage increased significantly till day 7, after which it gradually decreased till day 30 for all the test groups.

Discussion

Microleakage tests pertaining to the apical barrier may be regarded as contentious, as results may vary due to several variables.[7] The sealing ability of Biodentine and ESRRM putty has been researched and compared by various authors with contradictory results.[14,15] In literature, there is limited information on the apical leakage of MTA repair HP as a root-end filling material in an apexification model.[5] Thus, this study evaluated the sealing ability of MTA repair HP as a root-end filling material and compared its efficacy to that of Biodentine and ESRRM putty, for a period of 4 weeks.

Microleakage has been tested *in vitro* and *in vivo* using various techniques. Unfortunately, no accurate and consistent leakage test that meets all requirements is currently available.[16] An apical reaction can occur from coronal leakage of microorganisms into the canal from the pulpal chamber, which acts as a repository for bacteria.[2] In comparison to dye penetration or radioisotopes techniques, Mortensen et al.,[17] Krakow et al.,[18] and Jeevani et al.[19] stated that the bacterial microleakage technique seemed more accurate in leakage assessment and better simulated the clinical condition, as bacterial leakage is more relevant clinically. As a result, it is popularly used to appraise the sealing potential of root-filling materials.

The bacteria used in this study to evaluate bacterial leakage was *E. faecalis*, as it is the most frequently encountered organism

Table 1: Intergroup comparison of mean absorbance value using one-way ANOVA

	Absorbance value							
	At day 1		*At day 7*		*At day 14*		*At day 30*	
Groups	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Group I	0.0015	0.021	0.0035	0.0047	0.0025	0.0106	0.0019	0.0249
Group II	0.0004	0.0012	0.0024	0.0036	0.0021	0.0028	0.0017	0.0024
Group III	0.0008	0.0013	0.0028	0.0039	0.0024	0.0031	0.0018	0.0022
F-value *p*-value	3.017, 0.033, S*		0.984, 0.627, NS**		2.567, 0.056, NS**		1.254, 0.892, NS**	

*S, significant when $p < 0.001$; **NS, non-significant

Table 2: Intragroup comparison of mean absorbance value with repeated measures of ANOVA and *post hoc* test

	Absorbance value									
	At day 1		*At day 7*		*At day 14*		*At day 30*			
Groups	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*p-value*	*Post hoc pairwise comparison*
Group I	0.0015	0.021	0.0035	0.0047	0.0025	0.0106	0.0019	0.0249	<0.001	1 day < 7 days, 14 days, 30 days
Group II	0.0004	0.0012	0.0024	0.0036	0.0021	0.0028	0.0017	0.0024	<0.001	1 day < 7 days, 14 days, 30 days
Group III	0.0008	0.0013	0.0028	0.0039	0.0024	0.0031	0.0018	0.0022	<0.001	1 day < 7 days, 14 days, 30 days

in endodontic failures. This facultative, gram-positive, anaerobic bacterium can infiltrate deep into the dentinal tubules and can even survive in the absence of any nourishment.[2,20]

This study used a 4-mm apical plug similar to the study by Hachmeister et al.[21] and Bani et al.,[6] where they found a superior seal in a 4-mm plug irrespective of the biomaterials used.

In this study, ESRRM putty (group II) exhibited the least microleakage followed by MTA repair HP (group III) and the maximum microleakage was shown by Biodentine (group I). These differences within the groups were significant only on day 1 and not on days 7,14, or 30 (Table 1). This finding is in accordance with the studies conducted by Antunes et al.,[22] and Lagisetti et al.,[23] where they concluded that ESRRM putty and Pro Root MTA provided comparable sealing properties with ESRRM putty showing better but not significant results. This superior sealing efficacy of ESRRM which was attained may be attributed to the smaller particle size and the homogeneous consistency of the premixed material. The moisture in the dentinal tubules is sufficient for the material to set.[10] This eliminates the potential of heterogeneous consistency during mixing. The other attributing factor can be due to the higher pH which is attained earlier due to the lower setting time.[10]

Mineral trioxide aggregate repair HP performed better in this study when compared to Biodentine which could probably be due to its physicochemical formulation and faster initial setting time. The incorporation of a polymer plasticizer to MTA Repair HP's liquid structure results in a better putty and homogeneous composition that increases its plasticity during manipulation and creates a hydrated gel form that occupies the space between calcium silicate particles.[5] The fact that both MTA repair HP and ESRRM have an elevated pH which is sustained for a long time, as the material cures over several weeks, also supports our finding.[10]

It was also observed in this study, that the microleakage in all groups increased significantly till day 7 and then decreased till day 30, although the decrease difference was not statistically significant (Table 2). This finding could be attributed to the initial porosity, which is an inherent property of any tricalcium silicate-based cement and arises as an outcome of the spaces between the unhydrated cement grains. These spaces are water-filled, once the material hydrates and the hydration reaction advances, the hydration products occupy these gaps, and the porosity decreases. Thus, porosity is found to decrease as the cement ages.[10]

The overall results of this study showed no significant difference in the sealing efficacy among the three materials with time, which has some similarity to the findings of Juez et al.,[7] studies by Antony et al.[10] and Juez et al.,[7] compared the sealing ability of white MTA, Biodentine, and ESRRM putty as orthograde apical barrier materials and concluded that they all presented similar apical microleakage. Antony et al.,[10] compared the sealing ability of ProRoot MTA, Biodentine, and ESRRM putty as retrograde apical barrier materials and concluded that ESRRM putty and Biodentine presented similar apical microleakage whereas ProRoot MTA showed higher microleakage. The sealing ability of MTA repair HP has never previously been compared with any other cement besides NeoMTA Plus.[5] This is the first study that compares the sealing ability of MTA repair HP to ESRRM putty and Biodentine as an orthograde apical plug material. The results of this study showed that MTA repair HP showed similar sealing ability when compared to ESRRM putty and Biodentine. Hence, all three types of cement may be considered suitable as an orthograde apical plug material, although ESRRM putty and MTA repair HP have a handling advantage owing to their physicochemical formulations.

In this study, the limitations would include the technique sensitivity of the experimental setup, the preparation of the root apex by a Mani Peeso Reamer rather than ultrasonic retro tips might have produced more smear layer and negatively impacted the sealing ability of the material. Furthermore, the study being

an *in vitro* study cannot precisely emulate the clinical scenario. Nevertheless, additional research is required to validate the findings of this experiment and to scrutinize the behavior patterns of these materials *in vivo*.

Conclusion

Based on the findings of this study, it can be inferred that ESRRM putty, showed the least microleakage followed by MTA repair HP and Biodentine. The difference in microleakage was statistically significant only on the first day. With time, all three types of cement showed comparable results. The microleakage of all types of cement increased till day 7 following which there was a gradual reduction till day 30. These findings, thus, support their use as an orthograde apical plug material in immature permanent teeth. Additionally, MTA repair HP can be recommended for use as an orthograde apical plug material with a sealing ability similar to ESRRM Putty and Biodentine.

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