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Sealing Efficiency of MTA, Accelerated MTA, Biodentine and RMGIC as Retrograde Filling Materials

SUMMARY

Background/Aim: *This in-vitro study evaluated the apical-sealing ability of MTA, MTA+10%* $CaCl_2$*, Biodentine™ and RMGIC when used as retrograde material.* ***Material and Methods:*** *A total of 80 single-rooted bovine incisors were decoronated, instrumented, and divided into 4 groups according to retrograde material, as follows: Group 1: MTA (ProRoot MTA, Dentsply); Group 2: MTA (ProRoot MTA, Dentsply) + 10%* $CaCl_2$*; Group 3: Biodentine (Biodentine®, Septodont); Group 4: RMGIC (Nova Glass - LC, Imicryl). Root surfaces were isolated with nail polish, and teeth were immersed in 2% methylene blue dye at 37°C for 48 h. The extent of dye penetration (mm) was measured under a stereomicroscope.* ***Results:*** *RMGIC had the highest mean-rank dye penetration score (MP=49.05), followed by MTA (MP=43.65), Biodentine (35.95) and MTA+*$CaCl_2$ *(MP=33.35). The results of paired comparison tests found the mean microleakage value of MTA+10%* $CaCl_2$ *and Biodentine (Group 3) to be significantly lower than that of RMGIC (respectively; p=0,020, p=0,019). No significant difference was found in the other group comparisons (p> 0.05).* ***Conclusions:*** *These results suggest that the addition of an accelerator to MTA may reduce microleakage following endodontic surgery. Biodentine can be used as an alternative to MTA on retrograde obturation.*

Key words: Apical Microleakage, Apicoectomy, Biodentine, GIC, MTA, Root-end Filling Materials

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Introduction

Apical periodontitis is inflammation of the dental periapical tissue as a result of the colonization of microorganisms in the root-canal system[1]. Factors that may affect the etiology of the periapical pathology can be listed as the changes in the compound of root canal microbiota and host resistance to different types of microorganisms. The treatment of apical periodontitis is aims to remove the infection in the root-canal system and to relieve the symptoms[1]. Surgical endodontic treatments are an important option for teeth with apical periodontitis with a high failure rate with nonsurgical methods, or previously unsuccessful endodontic treatment[2].

The success of endodontic surgery is determined by numerous factors that include case selection, operator skill, and the properties of the retrograde material[3]. An ideal retrograde material should be biocompatible, stable, easily manipulated and provide a hermetic seal that prevents irritants from entering the root-canal space[4]. Mineral trioxide aggregate (MTA), which has a wide range of endodontic applications, was initially recommended as a retrograde filling material. MTA is a biocompatible, dimensionally stable material comprised of tricalcium silicate, tricalcium aluminate, tricalcium oxide, silicate oxide, tetra-calcium alumina ferrite and bismuth oxide and is known for its ability to induce hard-tissue formation[5-7].

MTA's superior sealing ability and biocompatibility are related to a physiochemical reaction in which calcium ions released from MTA react with phosphate ions in tissue fluids to form a hydroxyapatite-like precipitate[5-7]. Unfortunately, MTA has a delayed setting time that creates difficulties during apical surgery and leads to an increased risk of partial material loss and contamination[7,8].

$CaCl_2$ is a common setting accelerator that provides a significant, 50%-reduction in the initial setting time of cements[9,10]. The addition of $CaCl_2$ to MTA also improves sealing capacity, increases calcium release, and raises Ph[9,10]. Not only does the presence of $CaCl_2$ in the pores of MTA accelerate crystallization by increasing hydration to shorten setting time, it also significantly reduces maximum pore diameter and microleakage for up to 48 hours from the initiation of setting[9-12].

Biodentine™ is a calcium silicate-based biomaterial with physical and chemical properties similar to those of MTA. Like MTA, Biodentine can also be used in caries management, as a retrograde material, and for other endodontic procedures. Biodentine has good biocompatibility, sealing ability and color stability[13]. Moreover, the inclusion of calcium chloride and lack of bismuth oxide gives Biodentine a favourable setting time of 12-15 minutes[14]. Biodentine is reported to have mechanical properties similar to those of human dentin, and its solubility, structural strength and sealing capacity are similar to those of MTA. Compared to MTA, Biodentine offers ease of manipulation and a favorable setting time. Biodentine is claimed to have similar mechanical properties to human dentine, and its solubility, structural strength, and sealing capabilities are reported to be comparable to MTA[13].

Resin-modified glass ionomer cement (RMGIC) represents another clinical option for retrograde obturation[15]. RMGIC offer good chemical adhesion to tooth tissues, and when compared to MTA, it set faster, is less moisture-sensitive, and less soluble in oral fluids. Initial polymerization of RMGIC occurs through an acid-base reaction when the component powder and liquid are mixed together. This is followed by light polymerization in which a physical matrix is constructed that facilitates the chemical reaction to improve hardening and increase material resistance[15]. Histologic analyses that RMGIC is related with minimal inflammatory response and induces bone repair and formation of a connective-tissue lining when used as retrograde material[16].

The purpose of this *in-vitro* study was to evaluate the apical sealing ability of MTA, MTA+10% $CaCl_2$, Biodentine™, and RMGIC when used as retrograde material. The null hypothesis was that there would be no significant difference among these materials in terms of apical sealing ability, which was assessed using the dye-leakage method.

Material and Methods

Sample preparation

This *in-vitro* was conducted with 80 single-rooted, extracted bovine incisors with no cracks or calcification. After cleaning the external surfaces with curettes, teeth were decoronated using a diamond disc to create root specimens.

Root Canal Preparation

Access cavities were prepared and Gates Glidden drills were used for coronal flaring. Working lengths determined using a No. 25 K-file (Kerr, Romulus, MI). Root canals were prepared with the ProTaper rotary system (Dentsply/Maillefer, Baillagues, Switzerland) using the crown down technique and irrigation with 5.25% NaOCl and sterile saline solution. Root-canals were dried with paper points and obturated with laterally condensed gutta-percha (Dentsply/Maillefer, Ballaigues, Switzerland) and AH Plus sealer (Dentsply, De Trey, Konstanz, Germany). Excessive filling material was removed with an excavator, and access cavities were restored with adhesive system (Clearfil SE Bond®, Kuraray, Japan) and resin composite (Clearfil Majesty, Kuraray, Japan).

Apical Root Resection & Retrograde Cavity Preparation

The apical 3-mm portions of the teeth were resected using a diamond disk at a 90° angle to the long axis of teeth under water cooling. A 4-mm root-end cavity was prepared, using a periodontal probe to control cavity depth. Teeth were then randomly divided into 4 groups according to retrograde material. A plugger (Maillefer, Switzerland) with a rubber stop was positioned at the apical end of the root canal to aid in condensation and adaptation. All retrograde materials were applied according to the manufacturers' instructions as follows (Table 1):

Group 1 (n=20): ProRoot MTA was condensed and bulk-filled in the apical end of the root to a thickness of 4 mm. Samples were allowed to set in moist cotton pellet for 24 hours,

Group 2 (n=20): MTA with an addition of 10% $CaCl_2$ was condensed and bulk-filled in the apical end of the root to a thickness of 4 mm. Samples were allowed to set in moist cotton pellet for 24 hours,

Group 3 (n=20): Biodentine was condensed and bulk-filled in the apical end of the root to a thickness of 4 mm,

Group 4 (n=20): RMGIC was applied in 2 layers of 2 mm each to obtain a 4-mm thick filling in the apical end of the root. Each layer was polymerized for 20 seconds using a LED light at an intensity of 1200 mW/cm^2,

All teeth were stored at 37ºC in 100% humidity for 24 hours.

Table 1. Manufacturer, composition and clinical procedure information of the materials used in the study

Material	Manufacturer	Composition	Clinical Procedure
ProRoot MTA	ProRoot MTA, Dentsply, USA	Tricalcium silicate, bismuth oxide, dicalcium silicate, tricalcium aluminate, calcium sulphate dehydrate or gypsum	Powder and liquid are mixed in a ratio of 1/3.
Biodentine	Biodentine®, Septodont, Saint Maur des Fosses, France	**Powder:** tricalcium silicate, dicalcium silicate, calcium carbonate and oxide filler, zirconium oxide radiopacifier **Liquid:** Calcium chloride accelerator, water soluble polymer water reducing agent	Liquid and powder mixing at 4000–4200 rpm for 30 s.
Resin-modified Glass Ionomer Cement	R&D Series Nova Glass -LC, Imicryl, Turkey	**Powder:** Photosensitive glass powder, photo initiator **Liquid:** Polyacrylic acid, tartaric acid	1. Powder and liquid are mixed. 2. Light cured. for 20 s.
Dentin adhesive system	Clearfil SE Bond®, Kuraray, Japan	**Primer:** MDP, HEMA, Hydrophilic dimethacrylate, dl-Camphorquinone, Aromatic tert-amine, Water **Bond Liquid:** MDP, Bis-GMA, HEMA, Hydrophilic dimethacrylate, Photo initiator, Aromatic tert-amine, Silanized colloidal silica	1. Apply primer for 15 s 2. Dry 3. Apply adhesive 4. Light cure for 20 s.
Nanohybrid composite	Clearfil Majesty, Kuraray, Japan	Silanized barium glass filler, prepolymerized organic filler, Bis-GMA, hydrophobic aromatic dimethacrylate, di-camphorquinone	Light cure for 20 s.

Sealing Evaluation

Sealing ability of retrograde materials was assessed according to dye penetration test (Figure 1). The outer surfaces of the root specimens were covered with two layers of nail polish up to the apical 3 mm, and the specimens were immersed in 2% methylene blue dye at 37°C. After 24 h, the specimens were removed, cleaned, sectioned buccolingually, and evaluated under a stereomicroscope (Carl Zeiss-Jena, Germany) at x40 magnification by a single, calibrated examiner. Dye-penetrations scores were recorded according to the deepest level of penetration, as follows: 0: no dye penetration; 1: dye penetration between 0-1 mm, 2: dye penetration between 1-2 mm; 3: dye penetration between 2-3 mm.

Statistical Analysis

Data was analysed using the statistical software package IBM SPSS Statistics 16.0. Dye Penetration scores of the different filling groups were compared using Kruskal Wallis and Mann-Whitney tests. A level of $p<0.05$ was considered statistically significant.

Results

Dye penetration scores for each group are given in Table 2. The distribution of dye-penetration scores by group, the percentage of dye penetration scores for each group, and the mean ranks of dye penetration scores by group are shown in Figures 2, 3 and 4, respectively. As Figure 1, 2, and 3 shows, Group 4 (RMGIC) had the highest mean-rank dye penetration score (MP=49.05), followed by Group 1 (MTA, MP=43.65), Group 3 (Biodentine, MP=35.95) and Group 2 (MTA+%10 $CaCl_2$, MP=33.35).

*Table 2. Dye penetration scores of root-end filling materials. Mann Whitney U test, different superscript letters show statistically significant differences ($p<0.05$). * MR = Mean Rank*

Material	Score 0	Score 1	Score 2	Score 3	MR*
Group 1: MTA (n=20)	%25 (n=5)	%25 (n=5)	%20 (n=4)	%30 (n=6)	$43.65^{a,b}$
Group 2: MTA + %10 $CaCl_2$ (n=20)	%20 (n=4)	%60 (n=12)	%15 (n=3)	%5 (n=1)	33.35^{a}
Group 3: Biodentine (n=20)	%0 (n=0)	%90 (n=18)	%5 (n=1)	%5 (n=1)	35.95^{a}
Group 4: RMGIC (n=20)	%5 (n=1)	%45 (n=9)	%20 (n=4)	%30 (n=6)	49.05^{b}

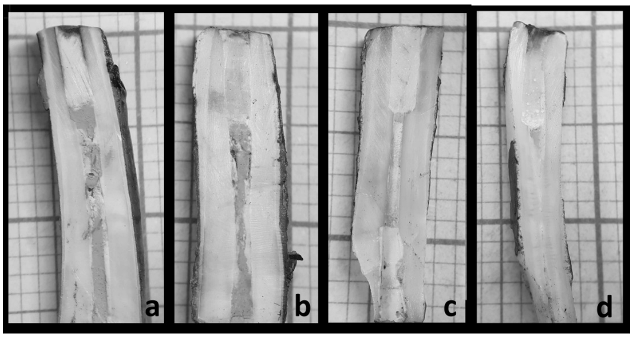

Figure 1. a) MTA - score 3, b) Biodentine - score 1, c) MTA + CaCl2 - score 0, d) RMCİS - score 2

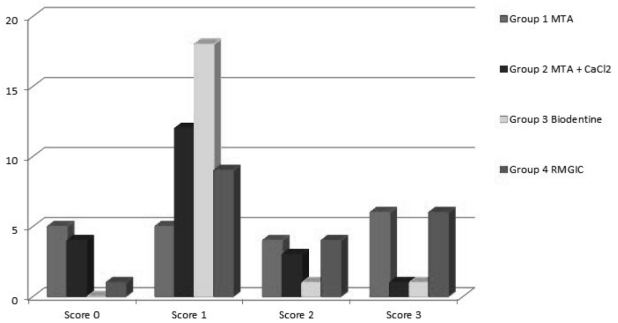

Figure 2. Dye penetration scores

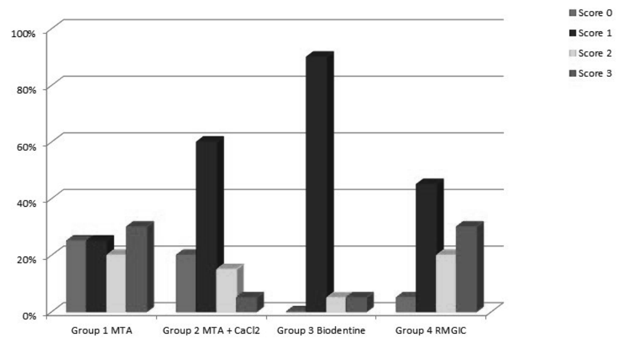

Figure 3. Percentage of dye penetration scores for each group

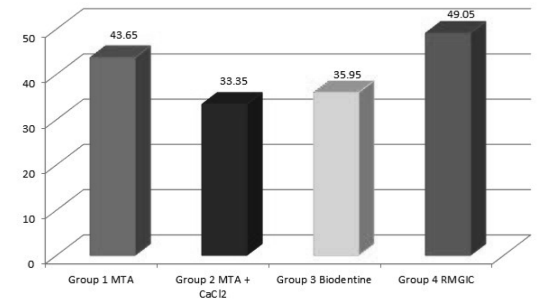

Figure 4. Mean-rank scores for dye penetration

The results showed a statistically significant difference in the mean-rank dye penetration scores of various groups. The best results were obtained the group with MTA+$CaCl_2$ (Group 2), but did not establish a statistically significant difference compared to MTA alone (Group 1) ($p>0.05$). The results of paired comparison tests found the mean microleakage value of MTA+10% $CaCl_2$ (Group 2) to be significantly lower than that of RMGIC (Group 4) ($p=0.020$). Similarly, the mean microleakage value of Biodentine (Group 3) to be significantly lower than that of RMGIC (Group 4) ($p=0.019$). Although, Biodentine samples (Group 3) had less dye penetration scores than that of MTA (Group 1), no statistically significant difference was observed between the groups ($p>0.05$). No significant difference was found in the other group comparisons ($p>0.05$).

Discussion

Despite the developments in endodontics, the apical microleakage yields the main reason of failures in endodontic treatment[17,18]. Microorganisms within the root canal can continue to survive in the dentinal tubules even though a strong mechanical-chemical preparation has been performed. The proliferation of microorganisms or endotoxins reaching the apical region can cause periapical tissue irritation and treatment failure, and an ideal closure of the apical region is desired to prevent this condition[17,19].

Endodontic surgical treatments are performed on very small and complex anatomical structures[18]. The success of this procedure is affected the root-canal anatomy, the physical and chemical properties of the retrograde materials, surgical techniques used, filling technique and, smear layer[18,19]. The most important criterion that affects the treatment results is the debriding and cleaning of the lateral canals and apical branches. This is closely related to the skill of the clinician and the use of modern equipment, which helps by examining the surgical field and provide comprehensive cleaning and preparation of the retrograde cavity. The other factors that may affect the prognosis of the endodontic surgical treatment are preoperative status of tooth, e.g. lack of a wide periapical lesion and placement of an ideal retrograde material to the root-end area[18].

The sealing ability of retrograde materials play important role in ensuring that bacterial products and periapical tissue fluids cannot penetrate the root-canal and create an environment conducive to bacterial replication[3-6]. In this *in-vitro* study evaluating the apical sealing ability of retrograde materials; RMGIC (Group 4; MP=49.05) had the highest mean-rank dye penetration score, followed by MTA (Group 1; MP=43.65), Biodentine (Group 3; MP=35.95) and MTA+$CaCl_2$ (Group 2; MP=33.35). The results of paired comparison tests found the mean microleakage value of MTA+10% $CaCl_2$ (Group 2) and Biodentine (Group 3) to be significantly lower than that of RMGIC (Group 4) (respectively; $p=0.020$, $p=0.019$); therefore, the null hypothesis was rejected.

In order to prevent retrograde microleakage at the root-end area, a retrograde cavity must be created during endodontic surgery[4]. Bani *et al.*[20] have stated that an apical plug of biomaterial with a thickness of 3-4 mm is sufficient to achieve apical sealing, and that the ideal retrograde cavity should be 4-mm deep and located in the centre of the root-canal parallel to the long axis of the tooth. In this study, a 4-mm root-end cavity was prepared and retrograde material was applied to a thickness of 4 mm.

The apical sealing ability of retrograde materials can be evaluated *in-vitro* using a number of techniques, including dye-leakage, liquid-leakage, radioisotope, SEM, electrochemical, and bacterial-leakage analysis[17]. Technically, there is no standardization in this regard and, small variations may arise even the same methodology is used[17]. In the dye leakage method, which is a passive technique to assess the apical leakage, the capillarity phenomenon is extremely important since the apex of the tooth is immersed in the paint that passes through any space between the canal walls and retrograde material [21]. According to Camps and Pashley [21], although the classical dye penetration method has limitations it offers the same results as liquid filtration and saves time in the laboratory. In the present study, apical sealing ability of materials was evaluated by immersing samples to 2% methylene blue dye for 48 h under a stereomicroscope at x40 magnification.

MTA's good marginal adaptability, a result of its hydrophilic structure[22], biocompatibility and 3-dimensional packing ability makes it the most preferred retrograde material[23]. Benz *et al.*[24] indicated that MTA presents superior results in terms of apical sealing when used as retrograde filling material. However, MTA has a number of drawbacks, such as discoloration potential, difficulties in manipulation, and delayed setting time, which entails the risk of the material being washed away by saliva and oral-tissue fluids during apical surgery[11,12,25]. The addition of $CaCl_2$ as an accelerator has been reported to improve the physicochemical properties of MTA, making it easier to handle and requiring less water in the mixing process. $CaCl_2$ also improves the compressive strength of MTA and reduces its solubility by making it less permeable[11,12,25].

Biodentine has been described as an innovative material offering good bioactivity and outstanding sealing properties[13,20]. Biodentine is easier to handle, mechanically stronger, and has a shorter setting time than MTA, and because it contains zirconium oxide rather than bismuth oxide as a radiopacifier, unlike MTA, it does not cause discoloration[13,14]. Whereas MTA's sealing ability is related to its hydrophilicity[7], Biodentine's sealing ability is attributed to its rich calcium and phosphate content[13]. SEM and confocal microscope images have shown both Biodentine and MTA to create tag-like structures within dentinal tubules and below the dentin-material interface that are instrumental in the materials' marginal sealing capacity and mechanical strength[26].

RMGlC is another material that has been used in retrograde material. RMGlC may be preferable in clinical practice because it is less moisture-sensitive and less solubility than other retrograde materials and because the clinician can control the initiation of polymerization[15,16]. The improved clinical outcomes of endodontic surgery performed using RMGIC have been associated with the material's biocompatibility and adherence to enamel and dentin[15,16]. While light polymerization helps improve RMGIC's fracture resistance under compressive and tensile stress, it may also increase the risk of polymerization shrinkage and subsequent microleakage over the long-term[27].

In the present study, the best results were obtained the group with MTA+$CaCl_2$ (Group 2). Although MTA+$CaCl_2$ (Group 2; MP=33.35) showed slightly less microleakage than Biodentine (Group 3; MP=35.95), there were no statistically significant differences between two groups ($p>0,05$). Similarly, the group with Biodentine (Group 3) had less dye penetration scores than that of MTA (Group 1; MP=43.65), no statistically significant difference was observed between them ($p>0.05$). These results are consistent with Bani et al.[20] and Mousavi *et al.*[28] studies which were reporting that MTA and Biodentine performed similarly in terms of apical microleakage[29]. In a similar *in-vitro* study which uses dye leakage method, it was indicated that Biodentine is statistically more successful compared to MTA derivates in terms of apical leakage [33]. Han and Okiji[32] noted that both Biodentine and MTA promoted Ca and Si uptake in the dentin adjacent to the root canal, although uptake was more pronounced with Biodentine than with MTA. In a recent study which uses liquid infiltration method, the micro-leakage results were reported to be similar to Biodentine[28]. Unlike them, Mandava *et al.* [29] reported MTA to possess superior sealing properties when compared to both Biodentine. Considering above mentioned literature and the findings of the current study, it can be said that Biodentine can be used as an alternative retrograde filling material to MTA.

According the present results, the samples with RMCIS had the highest dye penetration scores (MP=49.05). The significant difference was observed between the samples with RMCIS (Group 4) and MTA+10% $CaCl_2$ (Group 2) ($p=0.020$) and RMCIS (Group 4) and Biodentine (Group 3) ($p=0.019$). The results are in compatible with the findings of Kokate *et al.*[30] and Ravichandra *et al.*[31] found MTA and Biodentine to perform better than GIC in terms of microleakage values. Whereas, Costa *et al.*[15] reported that RMGIC provided similar marginal adaptability to that of MTA. Despite these results, we think that the apical sealing feature of RMCIS should be evaluated with more detailed methods.

Bortoluzzi *et al*.[9,10] evaluated the effect of addition of $CaCl_2$ to MTA on pH and calcium ion release. The results show that the addition of $CaCl_2$ to the MTA improves the physico-chemical properties of the material, the handling becomes more practical and lower amounts of water are needed in the mixing process. A study by Hong *et al.*[11] that evaluated microleakage of MTA with and without 10% $CaCl_2$ as an accelerator in an *in-vitro* apexification model concluded that the addition of an accelerator to MTA reduces microleakage and may be useful for endodontic surgery procedures. The results indicated that the addition of an accelerator to MTA may be beneficial in the one-session apexification procedure by reducing microleakage. Likewise, Harrington[12] reported that 10% $CaCl_2$ addition provides 50% reduction in the initial setting PC. Abdullah *et al*.[25] observed that adding $CaCl_2$ to cements appears to improve their sealing capacity and has no adverse effect on their biocompatibility or chemical properties. In the present study, MTA+$CaCl_2$ (Group 2) performed better than MTA alone (Group 1), but did not establish a statistically significant difference compared to the group with MTA (Group 1) ($p>0.05$). One possible explanation for the superior performance of the group with MTA+$CaCl_2$ may be related to the development of physico-chemical properties of the material and accelerated setting time achieved through the addition of $CaCl_2$. The addition of $CaCl_2$ as an accelerator can improve the sealing properties of the MTA and may benefit the endodontic surgery procedure.

Conclusions

Within the limitations of this study, MTA, MTA+$CaCl_2$, and Biodentine were all found to exhibit adequate sealing performance when used as retrograde filling material. The best results were obtained with MTA+$CaCl_2$, and Biodentine performed better than MTA alone. While Biodentine performed better than MTA alone, MTA's sealing performance may be improved by the addition of $CaCl_2$. However, clinical studies are required to support the findings of this *in-vitro* study.

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Animal Rights Statement: None required.

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