Original Article

Comparison of the sealing ability of apical plug materials in simulated open apices: An *in vitro* study

Ebru Delikan, Seçkin Aksu[1]

Department of Pediatric Dentistry, Faculty of Dentistry, Nuh Naci Yazgan University, Kayseri,
[1]Department of Pediatric Dentistry, Faculty of Dentistry, Mersin University, Mersin, Turkey

Abstract

Background: The aim of this study was to evaluate the microleakage of apical plugs made of four different materials in simulated immature teeth.

Materials and Methods: Ninety extracted single-rooted teeth were selected and randomly divided into six groups according to the materials used: NeoMTA, EndoSequence Bioceramic Root Repair Material Fast Set Putty (BC RRM-FS), Biodentine, Endo Repair, negative control, and positive control group. Microleakage was evaluated by using a stereomicroscope to assess the penetration of methylene blue solution. Data were analyzed using descriptive statistics and Pearson's Chi-square test ($P < 0.05$).

Results: EndoRepair group showed the highest leakage, and NeoMTA group exhibited the best sealing ability of apical plugs. The sealing ability of Biodentine was similar to NeoMTA and BC RRM-FS. On the other hand, the sealing ability of BC RRM-FS significantly lower than NeoMTA, but it provided a better sealing than EndoRepair.

Conclusion: According to the results of this study, NeoMTA and Biodentine can be used safely as apical plug material in single-session apexification in immature teeth. The BC RRM-FS, on the other hand, provided an acceptable sealing, although not as successful as the NeoMTA. However, the sealing properties of Endo Repair need to be improved.

Keywords: Apexification, biodentine, endo repair, microleakage, NeoMTA

Address for correspondence: Dr. Ebru Delikan, Department of Pediatric Dentistry, Faculty of Dentistry, Nuh Naci Yazgan University, Kayseri, Turkey.
E-mail: e.delikan@gmail.com

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INTRODUCTION

Since root development and apical closure continue after the eruption, root development may cease in the event of loss of pulp viability due to caries or trauma during this period. In these cases, successful endodontic treatment is challenging due to the wide root canal without an apical stop.[1] Moreover, different treatment protocols, such as traditional apexification, single-session apexification, or regenerative endodontic treatment can be used.[2-4]

In the traditional apexification method, calcium hydroxide is used to provide a physiological calcified tissue barrier in the immature open apices.[4] However, this method has many disadvantages.[5,6] Therefore, Morse *et al.*[7] presented a single-session apexification procedure for immature teeth. In this method, an artificial plug was formed at the immature apices, providing a hermetic seal. To date, many dental materials such as amalgam, mineral trioxide aggregate (MTA), calcium-enriched mixture (CEM) cement, Biodentine, etc., have been suggested as apical plug material.[8,9]

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Although the apexification procedure using MTAs has become the gold standard in immature teeth, it has some limitations such as tooth discoloration, poor handling, and prolonged setting time. Recently, the properties of MTA have been improved and these limitations have been tried to be eliminated. NeoMTA is a ground finer tricalcium silicate material that has tantalum oxide (Ta2O5) as radiopacifier. It can be easily manipulated and does not color the tooth.[10] However, there is no detailed information about the sealing ability of this material when used as an apical plug. In addition, there are newer calcium silicate-based materials such as Biodentine, Endosequence Bioceramic Root Repair Material Fast Set Putty (BC RRM-FS), which not cause discoloration and have good handling properties. Furthermore, they have a short setting time.[11] Endo repair, a pure calcium phosphate-based cement, was produced as an alternative to MTA. It has some advantages such as easy application, fast, and complete setting. The manufacturer has indicated that it can be used in direct and indirect pulp capping, apexification, root perforations, and vital pulpotomy.[12]

It is important to investigate the performance of the materials used as apical plugs in various applications and to present the results. In our literature research, no study was found evaluating microleakage when NeoMTA and Endo Repair were used as apical plug material. Therefore, the purpose of this study was to determine the microleakage of four biomaterials used as plug material in apexification treatments.

MATERIALS AND METHODS

Mersin University Noninterventional Clinical Research Ethics Committee approved this study (2018/167). Ninety single-rooted human permanent maxillary incisors, extracted due to periodontal problems, were used in this study. Periapical radiographs of the samples were obtained in buccolingual and mesiodistal directions to confirm Vertucci type I canal morphology. Teeth with severe caries, crack, root curvature, calcification, or developmental anomalies were excluded. The surfaces were cleaned, and the teeth were stored in 0.5% Chloramine T solution at 5°C.

The crowns were decoronated, and 2-mm root tip was removed with a high-speed diamond disc (Diabor, Istanbul, Turkey) under water cooling. The ultimate sample length was standardized to 12 mm. Root canals were shaped up to the Pro Taper Universal F5 file (Dentsply Maillefer, Ballaigues, Switzerland). The canal was irrigated with 5 mL 2.5% sodium hypochlorite and saline solution between instrument changes. Then, to create open apex, each specimen was prepared retrogradely using #4 Peeso Reamer drill (Dentsply Maillefer, Tulsa, OK, USA), corresponding to an apical diameter of 1.1 mm.[13] Final irrigation with 5 ml 2.5% NaOCl for 30 s with approximately 0.1 mL/s flow rate was performed and dried with sterile absorbent paper points (Dentsply, Maillefer, Switzerland). All instrumentation was carried out by the same operator.

In total, 90 samples were randomly divided into six groups of 15 samples each: (1) NeoMTA (Avalon Biomed Inc., Bradenton, FL, USA), (2) BC RRM-FS (Brasseler USA, Savannah, GA, USA), (3) Biodentine (Septodont, Saint Maur des Fosses, France), (4) Endo Repair (Hoffmann's Dental Manufactory, Wangenheim, Berlin, Germany), (5) Negative control, and (6) Positive control. The composition and the manufacturer of the materials used in the study are presented in Table 1.

Plug materials were condensed at a thickness of 3 mm to the apical portion using prefitted endodontic pluggers (Dentsply Maillefer, Tulsa, USA). A periapical radiograph was used to confirm the density, proper placement, and thickness. After the setting time recommended by the manufacturer, all canals were filled using a tapered gutta-percha (Dentsply Maillefer, Ballaigudes, Switzerland) and AH plus canal sealer (Dentsply, Detrey, Konstanz, Germany). Furthermore, if necessary, lateral condensation was performed. Coronal access was sealed using single bond universal adhesive (3M ESPE, Maplewood, MN) and universal composite resin restorative material (Filtek Z250, 3M ESPE). The external root surfaces except the 2 mm apical part were covered with two layers of nail varnish.

Positive control group samples were filled using AH plus canal sealer and gutta-percha and the coronal part of the root was closed with composite resin restorative material. No plug material was applied to the apical part. The external surfaces were rendered impermeable using two layers of nail varnish, except 2 mm part of the apical foramen. Negative control group samples were filled using AH plus canal sealer and gutta-percha, no plug material was applied to the apical part. The apex of the teeth was covered with sticky wax (Kerr, Berlin, Germany), and the outer surfaces, including apical foramen, were sealed using two layers of nail polish.

All samples were incubated at 37°C in 1% methylene blue dye for 48 h, then washed under tap water for 5 min. Subsequently, each tooth was sectioned vertically in buccolingual direction under water cooling with a slow-speed diamond saw (Isomet, Buehler, Lake Bluff, IL). Each specimen was examined under a

Table 1: Materials used in the study

Material	Composition	Manufacturer	Lot No
NeoMTA	Tricalcium silicate, dicalcium silicate, tantalum oxide, tricalcium aluminate and calcium Sulfate Water-based gel with thickener agents, water soluble polymers, proprietary ingredients	Avalon Biomed Inc., Bradenton, FL, USA	2017122201
BC RRM-FS	Tricalcium silicate, dicalcium silicate, calcium phosphate monobasic, calcium hydroxide, colloidal silica, water-free thickening agent	Brasseler, Savannah, GA, USA	1702FSPS
Biodentine	Tricalcium silicate, dicalcium silicate, calcium carbonate, iron oxide, zirconium oxide water	Septodont, Saint Maur des Fosses, France	B20212
Endo repair	Calcium phosphates, hydroxyapatite Distilled water (without preservatives)	Hoffmann's Dental Manufactory, Wangemheim, Berlin, Germany	8241
Filtek Z250	Bis-GMA, UDMA, Bis-EMA, TEGMA, zirconia, silika	3M ESPE, St Paul, MN, USA	N931611
Single bond universal adhesive	Bis-GMA, MDP, dimethacrylate resins, HEMA, Vitrebond copolymer, silane, ethanol, water	3M ESPE, St Paul, MN, USA	80912A

BC RRM-FS: Biocereamic Root Repair Material Fast Set Putty

stereomicroscope (Olympus SZ61, Munster, Germany) at × 40 [Figure 1]. A 12-mm ruler was placed next to the roots to aid in the evaluation of leakage rate under stereomicroscope. The degree of leakage was evaluated based on the penetration of the dye stain from apical to coronal of the root, and the dye penetration scores were created by modifying the method used in the study of De Moor and Hommez[14] [Table 2]. Two precalibrated researchers blindly scored all samples and disagreements were resolved through discussion.

The sample size was determined based on the results of Bani *et al.*,[2] aiming to obtain a power of 80%. SPSS version 22 (IBM, Corp, Armonk, NY, USA) was used to evaluate data. Qualitative results were identified using descriptive statistics, and Chi-square test with a Bonferroni correction was used to compare groups. The statistical significance level was $P < 0.05$.

RESULTS

During the application of the dye leakage test and the evaluation of the results, no sample was excluded from the study.

As shown in Table 3, all positive controls showed complete dye leakage, while the negative control group exhibited no leakage. There was a statistically significant difference between the positive and the negative control group ($P < 0.05$).

When the experimental groups were evaluated, 86.7% of the samples in the NeoMTA group, 53.3% of the samples in the Biodentine group, and 20% of the samples in the BC RRM-FS group showed no leakages While "score 1" leakage was observed in Biodentine, BC RRM-FS and NeoMTA groups (40%, 20%, 13.3%, respectively), "score 2" leakage was observed in BC RRM-FS and Biodentine (40%, 6.7%, respectively). The highest leakage (score 3) was observed in the Endo Repair and BC RRM-FS groups [100%, 20%, respectively, Table 3].

Table 2: Description of dye penetration scores

Score	Degree of dye penetration
0	No dye penetration
1	Dye penetration <1 mm
2	Dye penetration between 1-2 mm
3	Dye penetration >2 mm

If we examine the leakage in each group by evaluating Table 3 along the columns; "score 0" leakage was significantly different from the others (score 1, 2, and 3) in the MTA group. In BC RRM-FS group, "score 2" whereas in Biodentine group "score 0" and "score 1" significantly differed from other leakage degrees. This difference was seen in the Endo Repair group in "score 3."

Among the materials used as apical plug in the study, the highest leakage was seen in Endo Repair group and the least leakage was seen in NeoMTA group. When Biodentin was used as an apical plug, the sealing ability was not significantly different from NeoMTA but was significantly different from the negative control group. When used as apical plug, BC RRM-FS showed more microleakage than NeoMTA but less than Endo Repair. There was no statistically significant difference between BC RRM-FS and Biodentine leakage degrees [Table 3].

DISCUSSION

Necrotic immature teeth due to trauma or pulpal pathologies often have wide-open apex formation because of the interruption of root development. The thin dentin walls and the absence of apical constriction create difficulty in endodontic treatment.[15] In experimental studies, several different methods, such as overinstrumentation, retrograde instrumentation with NiTi rotary files, or sulfuric acid, have been used to simulate teeth with wide-open apexes.[16-18] In

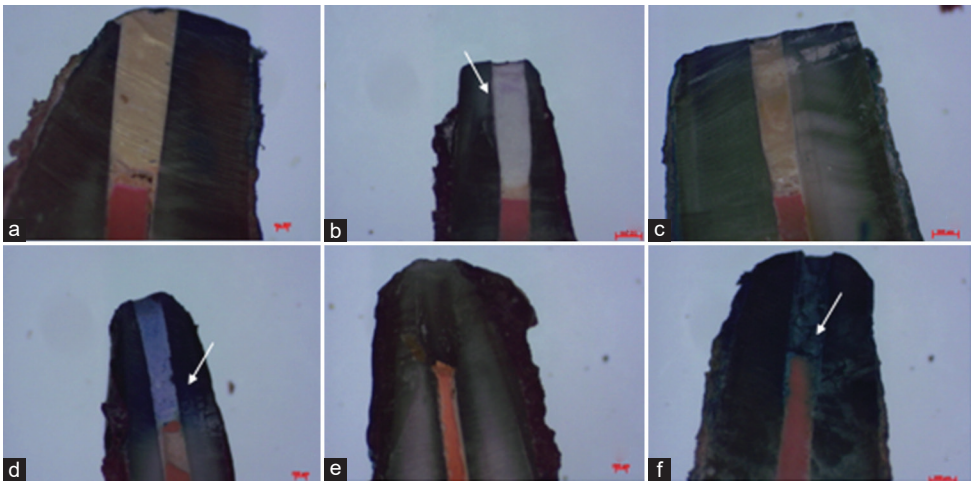

Figure 1: Stereomicroscope images of groups at × 40; (a) NeoMTA, (b) Biocereamic Root Repair Material Fast Set Putty, (c) Biodentine, (d) Endo Repair, (e) Negative control, (f) Positive control. White arrows show dye penetration levels

Table 3: Distribution of microleakage scores in the NeoMTA, BC RRM-FS, Biodentine™, Endo repair, and control groups

Microleakage	Materials, *n* (%)						Total (%)
	NeoMTA	BC RRM-FS	Biodentine	Endo repair	Negative control	Positive control	
Score 0	$13^{A,a,d}$ (86.7)	$3^{A,b,c}$ (20.0)	$8^{A,a,b}$ (53.3)	$0^{A,c}$ (0.0)	$15^{A,d}$ (100.0)	$0^{A,c}$ (0.0)	39 (41.4)
Score 1	$2^{B,a}$ (13.3)	$3^{A,a}$ (20.0)	$6^{A,a}$ (40.0)	$0^{A,a}$ (0.0)	$0^{B,a}$ (0.0)	$0^{A,a}$ (0.0)	11 (15.7)
Score 2	$0^{B,a}$ (0.0)	$6^{B,a}$ (40.0)	$1^{B,a}$ (6.7)	$0^{A,a}$ (0.0)	$0^{B,a}$ (0.0)	$0^{A,a}$ (0.0)	7 (10.0)
Score 3	$0^{B,a}$ (0.0)	$3^{A,a}$ (20.0)	$0^{B,a}$ (0.0)	$15^{B,b}$ (100.0)	$0^{B,a}$ (0.0)	$15^{B,b}$ (100.0)	33 (32.9)
Total	15 (16.7)	15 (16.7)	15 (16.7)	15 (16.7)	15 (16.7)	15 (16.7)	90 (100)

Data are given as number and percentage. Each different subscript lowercase specifies significant difference between raws ($P<0.05$). Each different subscript uppercase specifies significant difference between columns ($P<0.05$). BC RRM-FS: Biocereamic Root Repair Material Fast Set Putty

this study, immature teeth were simulated by applying NiTi files retrogradely.

The endodontic treatment of wide open apex teeth is challenging. In such cases, traditional apexification, single-session apexification, or regenerative endodontic treatment methods could be used.[2,3] Since traditional apexification has considerable disadvantages, such as increasing tooth fragility, extended treatment time (5–20 months), and re-infection risk due to the permeability of the temporary coronal restoration, the single-session apexification technique has become the more preferred approach.[6,19]

Microleakage is an important reason for the failure of single-session apexification treatments. Many variables, such as the filling technique used, the thickness of the plug, and the composition of the plug material, might influence leakage.[20] Thus, dimensional stability, adaptability, and the retentive ability of the plug material is important to seal the canal against the ingress of oral fluids and microorganisms. Different materials, such as Portland cement, a CEM, MTA, BC RRM-FS, and Biodentine have been suggested to constitute an artificial hard tissue barrier.[21] In the present study, the leakage associated with different plug materials that have been used in recent years were evaluated.

Various methods such as bacteria infiltration method, fluid filtration method, dye penetration method, radioisotope, and the electrochemical methods have been used to evaluate microleakage.[2,17,18,22] The dye penetration test is commonly used in studies because it is more economical and easier to apply than other methods.[23,24] Methylene blue (0.2%–2%), basic fuchsin (0.5%–2%), crystal violet (0.05%), aniline blue (2%), silver nitrate (50%), toluidine blue (0.25%), erythrocyte (2%), and rhodamine B (0.2%) are frequently used dyes in microleakage studies.[22,25,26] In the present study, methylene blue was preferred because of the ability to penetrate deeper along the canal than other dyes that it has a low-molecular-weight similar to that of bacterial products.[23]

The importance of the thickness of the apical plug material on the sealing ability was evaluated by Bani *et al.*[2] The 3-mm and 4-mm thickness were found more effective in preventing apical leakage than 1 mm and 2 mm thickness. These researchers suggested thickness of at least 3 mm MTA or Biodentine when used as apical filling material. Although a similar result was found for NeoMTA and Biodentine in the present study, it may be suggested to increase the thickness for BC RRM-FS and especially EndoRepair.

In the literature, many studies have investigated the leakage values of biomaterials when used as an apical plug.[2,20,27] According to the results of these studies, Biodentine has a comparable success rate to Angelus MTA. Although Angelus MTA has good sealing properties when used as an apical plug, Biodentine has good handling properties and short setting time.[2] Similarly, in this study, the sealing ability of NeoMTA was found higher than Biodentine, but there was no significant difference between them.

A study conducted in 2016 compared the marginal adaptation of various calcium silicate-based root filling materials. The quality of marginal adaptation has been identified as an appropriate criterion for evaluating sealability and resistance to leakage. No statistically significant difference was reported between the ProRoot MTA, NeoMTA plus, and BC RRM-FS.[11] The quality of marginal adaptation has been identified as an appropriate criterion for evaluating sealability and resistance to leakage. However, it has been noted that the application of a BC sealer to the canal before placing BC RRM-FS significantly increases the adaptation. In the current study, Biodentine and NeoMTA showed better sealing ability than BC RRM-FS. However, it is thought that sealing properties could be increased by using BC RRM-FS and BC sealer together.

Han and Okiji[27] evaluated the bioactivity of Endosequence BC sealer, Biodentine, and white MTA. They stated that the biological activity of the materials was related to the sealing ability. Endosequence BC sealer with lower Ca^{2+} ion release was reported to have a lower sealing ability than Biodentine and white MTA. In another study, Lermalapong *et al.* evaluated bacterial leakage of various bioceramics as apical plugin open apex model. ProRootMTA, Biodentine, TotalFill BC RRM paste, TotalFill BC RRM putty, and RetroMTA were used as an apical plug at 3 mm or 4 mm thicknesses. According to the results of the study, while both thicknesses of Biodentine and TotalFill BC RRM putty and the 4-mm ProRootMTA showed the best sealing ability, TotalFill BC RRM paste had the highest leakage for both thickness groups. It is thought that the different results regarding the sealing of the BC RRM sealant result from different material thickness, different test materials/techniques, and the presence or absence of sealant.

Different environmental conditions may have an effect on microleakage. For example, in a study, apical plug materials were placed in two different conditions: blood-contaminated and dry.[28] Microleakage was evaluated on the 1st, 4th, and 7th days. According to the results, more leakage was detected in plug materials placed in dry conditions. In the present study, plug materials were evaluated only in a dry environment, and leakage evaluation was performed only once (48th h). It cannot be determined how moisture and bleeding affect the results and whether there would be differences in leakage values over time. This can be stated as a limitation of the study. To overcome these limitations, new studies are needed to assess leakage in different environmental conditions and at different time intervals.

In literature, no studies have been found about Endo Repair, which has been reported as being used for apexification and root perforations by the manufacturer. Therefore, this study was performed to evaluate the apical sealing properties of NeoMTA, Biodentine, BC RRM-FS, and Endo Repair used as apical plug. According to the results, all material used as apical plug in the present study showed different amounts of leakage. NeoMTA showed the least leakage, while Endo Repair showed the highest leakage. The sealing ability of Biodentine appeared comparable with NeoMTA. BC RRM-FS leakage was significantly lower than that of NeoMTA but similar to Biodentine.

CONCLUSION

The results of this study suggest that NeoMTA and Biodentine may be used safely in single-session apexification in immature teeth. The BC RRM-FS, on the other hand, provided an acceptable sealing, although not as successful as the NeoMTA. The use of greater thickness or BC sealer can increase success. However, the sealing properties of Endo Repair need to be improved. The results of this study may help clinicians decide which apical plug material to use in open apices teeth. Further, *in vitro*, *in vivo*, and clinical studies are needed to verify our findings of microleakage from different plug materials.

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Conflicts of interest
There are no conflicts of interest.

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