RESEARCH

Apical microleakage of various biomaterials in simulated immature apices

Fatih Tulumbacı(0000-0003-0102-3223)[α], Volkan Arıkan(0000-0002-9331-0969)[β], Aylin Akbay Oba(0000-0001-8820-8671)[β], Işıl Sönmez Şaroğlu(0000-0002-3530-0244)[γ]

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ABSTRACT

Apical microleakage of various biomaterials in simulated immature apices

Background: In the treatment of open apex teeth, one-step apexification has been reported as an alternative method to the use of long-term calcium hydroxide.

Materials and Methods: The present study evaluated and compared apical microleakage when ProRoot MTA (Dentsply) (Group I), DiaRoot BioAggregate (Diadent) (Group II) and Biodentine (Septodont) (Group III) were used in the endodontic treatment of the teeth with simulated immature apices. To simulate immature teeth, 57 bovine maxillary incisor roots were prepared and randomly divided into three groups (n = 15 per group). The remaining teeth were included in negative (n=6) and positive (n=6) control groups. The materials were prepared according to the manufacturer's instructions and a radiograph was taken for each sample to confirm proper obturation of the samples. Apical microleakage was assessed using the dye penetration technique. Linear dye penetration was measured at 30X magnification and the data were analyzed using One-way ANOVA.

Results: Group I showed the lowest apical leakage with a mean dye penetration of 4.64 mm (±SD), whereas the leakage was highest in Group II with a mean dye penetration of 5.45 mm (±SD). The difference between the leakage in Group I and II was statistically significant ($p<0.05$), but the differences between other groups (Group I-III and Group II-III) were not ($p > 0.05$).

Conclusion: Based on the findings of this study, we conclude that the MTA and BD (especially AI) have the best sealing ability among the tested materials for treatment of obturated immature teeth.

KEYWORDS

BioAggregate, biodentine, endodontic treatment, microleakage, MTA

ÖZ

Olgunlaşmamış dişlerde kullanılan çeşitli biyomateryallerin mikrosızıntısının değerlendirilmesi

Amaç: Açık apeksli dişlerinin tedavisinde, uzun süreli kalsiyum hidroksit tedavisine alternatif bir yöntem olarak tek aşamalı apeksifikasyon yöntemi bildirilmektedir.

Gereç ve Yöntemler: Bu çalışmada, taklit edilen olgunlaşmamış açık apeksli dişlerin endodontik tedavisinde ProRoot MTA (Dentsply) (Grup I), DiaRoot BioAggregate (Diadent) (Grup II) ve Biodentine (Septodont) (Grup III) kullanıldığında dişlerde meydana gelen apikal mikrosızıntı değerlendirildi. Olgunlaşmamış dişlerin taklit edilmesi için, 57 sığır maksiller keser diş kökü hazırlanmış ve rasgele üç gruba ayrıldı (n = 15). Kalan dişler negatif (n = 6) ve pozitif (n = 6) kontrol grubuna dahil edildi. Malzemeler, üretici firma talimatlarına göre hazırlandı ve örneklerin uygun şekilde doldurulduğunu doğrulamak için her örnekten bir radyograf alındı. Apikal mikrosızıntı, boya nüfuz etme tekniği kullanılarak değerlendirildi. Doğrusal boya penetrasyonu 30X büyütmede ölçüldü ve veriler ANOVA kullanılarak analiz edildi.

Bulgular: Grup I, ortalama boya penetrasyonu 4.64 mm ile en düşük apikal sızıntıyı gösterirken, sızıntı Grup II'de 5.45 mm boya penetrasyonu ile en yüksekti. Grup I ve II'deki apikal sızıntı arasındaki fark istatistiksel olarak anlamlı ($p <0.05$), ancak diğer gruplar arasındaki istatistiksel farklar (Grup I-III ve Grup II-III) anlamlı değildi ($p> 0.05$).

Sonuç: Diğer materyaller ile karşılaştırıldığında MTA VE BD içeriklerindeki farklılıklar nedeniyle (özellikle AI) olgunlaşmamış apeksli dişlerin tıkanmasında kullanıldığında en iyi sızdırmazlık kabiliyetine sahip olduğunu düşündürmektedir.

ANAHTAR KELİMELER

BioAggregate, biodentine, endodontik tedavi, mikrosızıntı, MTA

Following the eruption of teeth, root development and closure of the apex continues for approximately 3 years.[1] When an injury damages the Hertwig's root sheath or when pulp necrosis occurs because of infection or trauma, root development and closure of the apex may be arrested.[2] When endodontic treatment is required in immature teeth, some challenges arise because of the incomplete closure of the apex and thin dentinal walls. For example, achieving a proper obturation of the canal and a

[α] Ankara Yıldırım Beyazıt University Faculty of Dentistry, Department of Pediatric Dentistry, Ankara, Turkey
[β] Kırıkkale University Faculty of Dentistry, Department of Pediatric Dentistry, Kırıkkale, Turkey
[γ] Adnan Menderes University Faculty of Dentistry, Department of Pediatric Dentistry, Aydın, Turkey

hermetic seal is difficult in immature teeth due to the wide opening in the apical area.[3,4] In root canal treatment of immature teeth, another issue is the thin dentinal walls that are reported to predispose these teeth to a higher incidence of root fractures.[5]

Traditionally, apexification has been used for the treatment of immature teeth.[4] Researchers reported a success rate of 79-96 % for long-term calcium hydroxide treatment.[6] Calcium hydroxide has various advantageous biological properties such as antimicrobial activity, tissue dissolving ability, inhibition of tooth resorption, induction of repair by hard tissue formation and alkaline pH (pH 12.5).[7]

In recent years, another treatment concept, namely single-step apexification, has become popular for the treatment of teeth with immature apices. In this method, the apex is occluded with a biocompatible material; thus, an artificial apical stopper is formed that allows filling of the root canal.[8]

Mineral trioxide aggregate (MTA) was the first material used in single-stage apexification treatment.[9,10] MTA has a good root sealing ability, low cytotoxicity and high degree of biocompatibility and also aids the formation of apical hard tissue.[10,11] The material consists of thin hydrophilic particles of tricalcium silicate, tricalcium oxide and silicate oxide, and it shows an alkaline reaction in aqueous slurries (pH>11). MTA is highly recommended for apical retrograde root filling, apexification and pulp capping[11-13] nevertheless, it has certain disadvantages, such as a long setting time, difficulty of manipulation, low resistance to compression and flow capacity, discoloration of tooth, presence and release of arsenic and high cost.[14,15] New calcium silicate-based materials have recently been developed as alternatives to MTA.[16,17]

BioAggregate (BA) is a relatively new bioceramic-based material similar to MTA.[18] The material consists of calcium silicate, calcium hydroxide, hydroxyapatite, tantalum oxide and silicon oxide. BA differs from MTA because it is aluminium-free, which reduces the toxic effect to human cells, and it contains a significant amount of tantalum oxide instead of bismuth oxide as a radiopacifier.[17] BA is bioactive, biocompatible with human periodontal ligament fibroblasts and has similar antibacterial effects to MTA against Enterococcus faecalis.[17,19] BA is used for retrograde root filling, perforation repair, vital pulp therapy and induction of apical closure in incompletely developed teeth.[4]

Biodentine (BD) has been developed as a permanent dentine substitute material for use when the original dentine is damaged. It has dentin-like mechanical properties and can be used on both crowns and roots.[20] As a powder, it mainly contains tricalcium silicate, dicalcium silicate and the principal component of Portland cement. As a liquid, it contains calcium chloride, which is an accelerator and hydrosoluble polymer.[17] BD has been shown to be biocompatible with human gingival fibroblasts; it is also bioactive, has a low setting time, a good sealing ability and is less cytotoxic than other materials currently used in pulpal therapy. Considering these superior properties, BD is a potential material for use in one-visit apexification procedures.[21]

MTA and calcium hydroxide are still commonly used materials for apexification in immature teeth; however, studies evaluating the success of BA and BD as filling materials in one-step apexification are currently lacking. Therefore, the present *in vitro* study aimed to compare MTA, BA and BD as root-end filling materials, particularly in relation to their success in preventing microleakage. Hypothesis tested was that permanent dentine substitute material (BD) is expected to have more positive results than the other materials.

MATERIALS AND METHODS

This *in vitro* study was conducted in, Department of Pediatric Dentistry, Faculty of Dentistry, Kırıkkale University, Turkey over a period of six months. In total, 57 extracted bovine maxillary incisors with similar root dimensions (Bucco-lingual: 7.50±1 mm; Mesio-distal: 4±1 mm) were selected for use in the present *in vitro* study. Following extraction, the teeth were cleaned of debris with curettes and stored in normal saline. Crowns and apical parts of the roots were sectioned with a low–speed diamond disk saw under water coolant to obtain 12-mm-long standard root samples (Figure 1). The canals of the samples were prepared up to #100 K files (Dentsply Maillefer, Ballaigues, Switzerland). The canals were irrigated between instruments with 2 ml of 2.5 % sodium hypochlorite (NaOCl). To simulate open apices, apical parts of the samples were prepared up to # 6 Gates Glidden drills (Lexicon®, Dentsply, Tulsa Dental, Tulsa, OK, USA) and the blunderbuss form was given. For the confirmation of the blunderbuss form, each sample was controlled with a periapical radiograph. Finally, the root canals were irrigated with 5 ml of 2.5 % NaOCl followed by 5 ml of 17 % Ethylenediaminetetraacetic acid solution and 5 ml saline. Following root canal preparation, paper points were used to dry the canals. The samples were randomly divided into three experimental groups each comprising 15 teeth. The specimens were then obturated (with plugger) as follows: Group I with ProRoot MTA (Dentsply, Tulsa, OK, USA), Group II with DiaRoot BA (Diadent, Burnaby, Canada) and Group III with BD (Septodont, Cedex, France). The remaining teeth were included in negative (n = 6) and positive (n = 6) control groups (Figure 2).

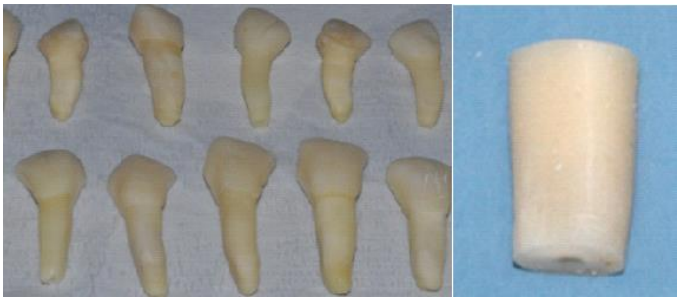

Figure 1.

Bovine teeth and standard root samples (12- mm)

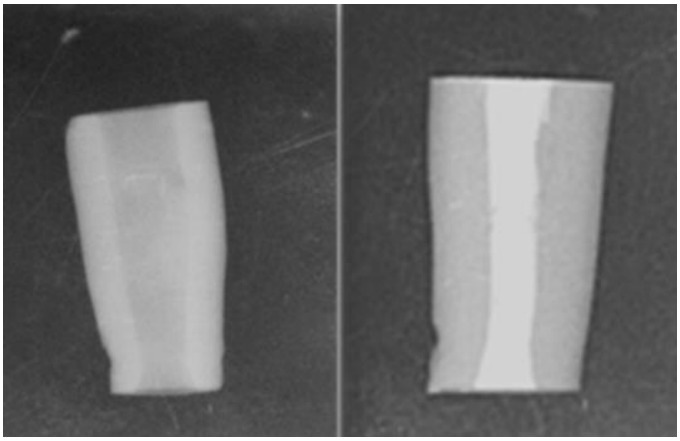

Figure 2.

Prepared and filled samples

After the placement of moist cotton pellets over the apices, the materials were prepared according to the manufacturers' instructions and placed into the canals using pluggers. A radiograph was taken for each sample to confirm proper obturation of the samples. Specimens were wrapped in a piece of wet gauze and placed in an incubator for 48 h at 37 °C and 100 % relative humidity. The samples were covered with two layers of nail varnish and sticky wax, except for 2 mm of their apical parts, and they were immersed and maintained in 2 % methylene blue dye for 72 h. In the positive controls group; samples were not filled with any filling materials and not covered with two layers of nail varnish and sticky wax. In the negative control group; samples were not filled with any filling materials but, were covered with two layers of nail varnish and sticky wax.

After 72 h, the samples were rinsed with running water and the varnish and sticky wax were removed using a scalpel blade. The samples were sectioned longitudinally with the help of guide grooves that were prepared on the buccal and lingual sides of the roots using diamond discs under copious irrigation with cold water (Figure 3). The microleakage was determined by measuring the distance in millimeters between the apical plug and the last point of leakage in the canal on 30X magnified images using Image Focus 4.0 (Euromex, Arnhem, Germany).

Figure 3.

Longitudinal section sample

Statistical analysis

Statistical analysis, was conducted using Statistical Package for Social Sciences (SPSS) version 17.0 (SPSS Inc., Chicago, IL, USA). The means and standard deviations (SD) were calculated and differences were analyzed using one-way analysis of variance (ANOVA) and Tukey's test. $P<0.05$ was considered statistically significant.

RESULTS

In the negative control group, no leakage was observed; in the positive control group, all canals were stained. Table 1 shows the mean microleakage values and SD for all groups. The BA group showed the highest microleakage scores (5.453 ± 0.608), whereas the MTA group showed the lowest microleakage (4.640 ± 0.789); furthermore, there was a significant difference between the microleakage scores in these groups (one-way ANOVA: $p=0.007$). Multiple comparisons showed that the difference between MTA and BA was statistically significant ($p<0.05$), but the differences between the other groups were not ($p>0.05$) (Table 2).

Table 1.

Comparison of microleakage (mm) for the materials used in the study

Descriptive statistics

Microleakage	N	Mean	SD	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
MTA	15	4.640	0.789	4.203	5.077	3.2	5.9
BioAggregate	15	5.453	0.608	5.117	5.790	4.1	6.2
Biodentine	15	4.977	0.600	4.645	5.309	4.1	6.1
Total	45	5.023	0.738	4.802	5.245	3.2	6.2

Table 2.

Multiple comparisons of microleakage (mm) values among the three groups

Dependent Variable: Microleakage					
(I) Group	(J) Group	Mean Difference (I-J)	P	95% Confidence Interval	
				Upper Bound	Lower Bound
MTA	Biodentine	0.337	0.397	0.959	0.285
MTA	BioAggregate	0.813	0.008	1435	0.191
Biodentine	BioAggregate	0.477	0.164	1099	0.145

* *The mean difference is significant at the 0.05 level*

DISCUSSION

Calcium hydroxide has been used in apexification treatments for many years; however, this concept of treatment has several disadvantages including the variable treatment times, unpredictable formation of an apical seal, difficulties in patient follow-up, delays in treatment, increased risk of root canal reinfection and reduction in fracture resistance.[22-24] Considering these disadvantages, the formation of an MTA apical plug in a single visit has, in recent years, been suggested as an alternative method to apexification with calcium hydroxide for the treatment of nonvital immature permanent teeth.[10,12] The advantages of one-visit apexification are as follows: shortened treatment time; possible immediate restoration of the tooth and, thus, prevention of root fracture, which can sometimes occur during long term apexification with calcium hydroxide; and prevention of changes in the mechanical properties of dentine because of the prolonged use of calcium hydroxide.[1,25]

MTA has positive features such as the prevention of microleakage, antibacterial effects, high marginal adaptation, alkaline pH and biocompatibility.[11] It is a bioactive material that stimulates the production of interleukins and cytokine release and is capable of promoting hard tissue formation.[26] However, the long setting time of the material is a major shortcoming, as are its difficult handling characteristics, discoloration potential (grey MTA), low washout resistance and high cost.[15]

BD is a good alternative to MTA.[27-29] The content of this biomaterial is very similar to MTA (with the exception of zirconium oxide, which is added to BD powder).[30] The setting time of BD is relatively short (12 min); thus, root canal obturation can be performed in the same appointment. Consequently, apexification with BD requires less time than with MTA, and this reduces the risk of fractures for immature teeth with thin roots.[31]

BA, which shows similar biomineralizing characteristics and Ca+2 release to MTA, is another alternative material that can be used for apexification, repair of root perforation and resorption and vital pulp therapy.[19,32] BA contains a significant amount of tantalum oxide instead of bismuth oxide as a radio-pacifier, which is the major difference between BA and MTA.[19] Tantalum oxide works as a strong inhibition zone when osteoblasts are grown, whereas fibroblasts proliferate well on a tantalum disc.

The three materials tested here all have potential for use in one-visit apexification; however, studies comparing these materials are currently lacking and no previous studies have compared them in the apexification of immature teeth. Therefore, our study was designed to compare MTA and its potential alternatives for their success in the apical sealing of immature teeth.

In the present study, the methylene blue dye penetration method was used because of its low cost, ease of manipulation, high degree of staining and lower molecular weight than that of bacterial toxins.[33,34] However, the *in vitro* penetration of dye into canals should not be considered directly comparable with the *in vivo* leakage of irritants from the root canal system. Methylene blue is used in microleakage studies because the filling material that allows for the penetration of small molecules (e.g. the dye) has the potential to prevent leakage of larger substances (e.g. bacteria and their byproducts).[33,35] So that, the teeth were cut longitudinally to determine how far the leak had taken.

MTA is a type of hydraulic cement that is set in the presence water.[30] In the apical

region, after forming an initial mechanical seal, MTA continues to dissolve and this results in the formation of hydroxyapatite-like crystals between the material and canal walls; hence, MTA shows superior adhesion that prevents the penetration of dye and results in low microleakage.[36,37] Congruently, in an *in vitro* study that compared dislodgment resistance of MTA and BA in furcation perforations, Hasheem & Amin reported that the bond strength of BA was significantly lower than that of MTA. In the present study, BA showed the highest microleakage compared to the other tested materials. MTA has long-lasting hydration reactions and maturation duration.Calcium hydroxide and calcium silicate hydrate gel are the main hydration products. Calcium hydroxide is suggested to function in the formation of the hydroxyapatite-like structure. Calcium phosphate monobasic, which is included in the composition of BA instead of tetracalcium aluminaferrite to avoid discoloration, has been suggested to play a role in HA coprecipitation, decreasing calcium hydroxide crystals and reducing the bond strength and sealing ability of BA.[38]

According to the results of the present study, there was no statistical difference in microleakage when MTA and BD were used. Because BD has similar content to MTA (except for zirconium oxide, which is added to the powder, and a setting accelerator water reducing agent, which is added to the liquid)[30], this result is expected. However, this result is incompatible with the results of Nanjappa et al (2015), who showed that BD had a better sealing ability than MTA. They stated that the slow setting reaction of MTA might be the reason for the increased depth of microleakage.[31] A similar difference was not observed in our study. This discrepancy may be a consequence of the difference between the methods used. For example, they filled the root canals using gutta-percha and AH-Plus and prepared apical area afterwards; therefore, remnants of AH-Plus that could not be removed from the canal walls may have led to results that were different from ours. Bani et al. (2015) reported similar results to those in our study: they observed no significant difference between the microleakage values of MTA and BD. Because BD has favorable handling characteristics and its placement is less time-consuming than MTA, it is used as an orthograde apical filling in teeth with open apices.[30] Similarly, Butt et al. investigated the sealing ability of mineral trioxide aggregate and BD in immature apices. They found no significant difference between the microleakage values of MTA and BD and reported that BD exhibits similar properties to MTA.[32] In both materials, a calcium silicate hydrate colloidal gel layer and apatite deposits form on the surfaces, which interact with tissue fluids and enhance the sealing ability of the materials.[27] Alsubait et al (2014) also compared the push-out bond strength of ProRoot MTA, BA and BD and reported that there was no significant difference between White MTA (WMTA) and BD. They reported that BA showed significantly lower bonding strength when compared to both WMTA and BD.[17] Researchers have explained the higher retention in WMTA and BD as a result of the tag-like structures formed in the adjacent root canal dentin[33], and the low push-out bond strength of BA has been attributed to the lack of aluminium in this material.[17]

Complete delivery of apical stop barrier has significant clinical effects on the long-term prognosis of treatment. MTA and BD have given positive results *in vitro* studies and are biomaterials that can be used for this purpose in the treatment of open apex teeth. Clinical trials are needed to see the long-term results of this biomaterials.

CONCLUSION

Within the limitations of this study (low molecular weight of methylene blue, which results in deeper penetration compared to bacterial toxins, and the differences between bovine and human teeth), our findings suggest that for obturation of immature apices, MTA and BD shows the best sealing ability. Therefore, these two materials could be used as viable alternatives to each other in the treatment of immature teeth with open apices.

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Corresponding Author:

Assist. Prof. Fatih TULUMBACI
Ankara Yıldırım Beyazıt University
Faculty of Dentistry
Department of Pediatric Dentistry
Ankara, Turkey
Phone : +90 312 324 15 55
Fax : +90 312 324 15 05
E-mail : fatihtulumbaci@hotmail.com