Original Article / Özgün Araştırma

Comparison of Microleakage and Root Canal Dentin Adaptation After Single Visit Apexification Treatment with Retrograde Filling Materials in Single Rooted Young Permanent Teeth

Tek Köklü Genç Daimi Dişlerde Retrograt Dolgu Materyalleri ile Tek Seans Apeksifikasyon Tedavisi Sonrası Mikrosızıntı ve Kök Kanal Dentinine Adaptasyonun Karşılaştırılması

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Abstract

Objective: The aim of this study was to evaluate coronal and apical microleakage and dentin adaptation of three different types of mineral trioxide aggregate (MTA) and Biodentine using scanning electron microscope (SEM) in single visit apexification procedures carried out *in vitro*.

Materials and Methods: A single visit apexification treatment was applied to the 200 single-rooted permanent teeth with the use of Angelus MTA, Ortho MTA, Retro MTA and Biodentine as 20 samples in each group. Coronal and apical leakage was assessed for each group; positive and negative control groups. For the microleakage test, samples were incubated in methylene blue, and then measured under a stereomicroscope. Sixteen specimens were prepared for evaluating the dentin adaptation. SEM examinations were performed by measuring micrometric measurements of the material and dentin spaces on the images of the apical and coronal regions. The obtained data were analysed statistically.

Results: According to the microleakage evaluation, apical and coronal measurements indicated significantly more leakage than Biodentine Angelus MTA and Retro MTA, but no significant difference was found to be between MTA groups ($p<0.05$). While there was no significant difference in the apical region between the groups dentin adaptation; in the coronal region, Biodentine was found to have more gap dimensions at the significant level than the other groups ($p<0.05$).

Conclusion: Biodentine was not as successful as the other groups in the microleakage and marginal adaptation.

Öz

Amaç: *İn vitro* olarak 3 farklı mineral trioksit agregat (MTA) materyali ve Biodentine'in tek seans apeksifikasyon uygulamalarında koronal, apikal mikrosızıntı ve taramalı elektron mikroskobu (SEM) ile dentin adaptasyonu değerlendirmesi hedeflenmiştir.

Gereç ve Yöntemler: Her grupta 20 örnek olacak şekilde toplam 200 tek köklü daimi dişte Angelus MTA, Ortho MTA, Retro MTA ve Biodentine kullanılarak tek seans

apeksifikasyon tedavisi uygulanmıştır. Her grup ve pozitif, negatif kontrol grupları için koronal ve apikal sızıntı değerlendirilmiştir. Mikrosızıntı testi için örnekler metilen mavisi içerisinde bekletildikten sonra stereomikroskop altında ölçümler yapılmıştır. Dentin adaptasyonunun değerlendirilmesi için 16 örnek hazırlanmıştır. SEM incelemesi ile apikal ve koronal bölgelerdeki görüntüler üzerinde materyal ve dentin arası boşluklar bilgisayar ortamında mikrometrik olarak ölçülerek yapılmıştır. Elde edilen veriler istatistiksel olarak analiz edilmiştir.

Bulgular: Mikrosızıntı değerlendirmesi sonuçlarına göre apikal ve koronal ölçümlerde Biodentine Angelus MTA ve Retro MTA'ya göre anlamlı derecede daha fazla sızıntı gösterirken, MTA grupları arasında anlamlı bir fark bulunmamıştır ($p<0,05$). Dentin adaptasyonu açısından apikal bölgede gruplar arası anlamlı bir fark görülmezken, koronal bölgede Biodentine diğer gruplara göre anlamlı derecede daha fazla boşluk boyutuna sahip grup olarak saptanmıştır.

Sonuç: Biodentine'nin mikrosızıntı ve marjinal adaptasyon açısından diğer gruplar kadar başarılı sonuçlar veremediği görülmüştür.

Introduction

Young permanent teeth, traumas, dental anomalies, and deep caries that damaged the pulp tissue may lose its vitality, and pulp necrosis causes the cessation of root development. Endodontic treatments are needed during this stage; however, thin and fragile dentin walls, wide canals, and apex morphology make the endodontic treatment difficult. In the treatment of young permanent teeth, a root canal filling should be made by closing the apex with natural or artificial barriers. Some treatment procedures also aimed at maintaining the root development and regeneration of pulp tissues.

Traditional apexification treatments have yielded successful results recently. However, long treatment sessions and repeated visits led to the search for alternative treatments. One of these alternatives, regenerative endodontic therapy, comes to the forefront with practical application and the use of easy-to-reach materials. In addition, retaining the tooth with tissues free from immune system reactions and that does not cause pathological responses is preferable (1).

The single visit apexification method is a treatment based on the principle that the open apex is covered with a biocompatible material to fill the canal (2). Unnecessary prolonged treatment processes are regarded as advantages in this method (3). Single visit apexification treatment was first described using a mineral trioxide aggregate (MTA). Various MTA preparations have been developed to overcome the adverse effects, such as long curing time and discoloration over time, which are the advantages of MTA in endodontic treatment (4).

One of the goals of root canal filling is to obtain a hermetic seal in the root canal system to support healing after endodontic treatment. Inadequate filling and failure to provide the apical plug may cause fluid penetration due to the periapical chronic inflammatory reaction and may prevent the success of the treatment (5). As young permanent teeth morphologically differ, leakage after endodontic treatment also poorly affects the prognosis.

This study aimed to evaluate the dentin adaptation with three different MTA and Biodentine materials *in vitro* with coronal, apical microleakage, and scanning electron microscopy images (SEM).

Materials and Method

This study was approved by the Aydın Adnan Menderes University Medical Faculty Non-Invasive Clinical Research Ethics Committee on 14 June 2016 with the decision number 25.

A total of 20 teeth were selected in each experimental group, with a total of 160 teeth, and 8 and 16 teeth in the positive and negative control groups, respectively (Table 1). A total of 16 dental SEM examinations were planned, with four materials applied for each SEM.

The study included permanent teeth indicated for extraction due to orthodontic reasons in patients aged between 12 and 18 years who visited the Department of Dentistry Faculty in Aydın Adnan Menderes University. Decayed, fractured, cracked, restored, open apex and root resorbed permanent teeth were not included in the study. The teeth were stored in 5.25% NaOCl for 1 hour (h) after removal from the soft tissue using curettes. Samples were stored in the sterile saline solution at room temperature for approximately 8 weeks until being used in the study.

The root lengths of the teeth used in the study were shortened to 12 mm. The traditional canal cavity was shaped on the teeth. The apical portions of the

Table 1. Experimental and control groups

	N	Investigated region	Material
Group 1	20	Apical	Angelus MTA tricalcium silicate, dicalcium silicate, tricalcium aluminate, calcium oxide, calcium tungsten, bismuth oxide
	20	Coronal	
Group 2	20	Apical	Retro MTA calcium carbonate, silicon dioxide, aluminium oxide, calcium zirconia mixture
	20	Coronal	
Group 3	20	Apical	Ortho MTA tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetracalcium aluminoferrite, calcium oxide, bismuth oxide
	20	Coronal	
Group 4	20	Apical	Biodentin tricalcium silicate, dicalcium silicate, calcium carbonate, calcium oxide, iron dioxide, zirconium dioxide
	20	Coronal	
Positive control	4	Apical	--
	4	Coronal	
Negative control	8	Apical	Angelus MTA, Retro MTA, Ortho MTA, Biodentin
	8	Coronal	

MTA: Mineral trioxide aggregate

samples were expanded using 1-6 numbered Gates-Glidden drills (JS Dental, Ridgefield, USA), and the root morphology of open apex teeth was simulated. In the canal, preparations were made with files (Golden Star Medical Co., Shenzhen, China). The coronal and middle sections were enlarged with circumferential inclination until the #80 file easily reached the apex. The root canals were irrigated with 2 mL of 2.5% NaOCl (Kim San Medicine, İstanbul, Turkey). After preparing the root canal, irrigation was performed with 5 mL of 2.5% NaOCl and 5 mL of sterile saline solution. Root canals were dried using paper points (Diadent, Diadent Group International, Burnaby, BC, Canada).

The prepared samples were filled with paperpoint so as not to overflow from the apical part, and the top of the apical part was covered with modeling wax. Subsequently, the paper points were removed and the canals were filled with the test materials prepared in accordance with the manufacturer's instructions. The canals were filled by condensation. However, no canal filling was applied in the positive control group; the same canal filling procedure with two coronal and apical evaluation of each material was performed in the negative control group. Radiographs were taken from the canal filling samples and checked.

The samples in the experimental and positive control samples, which will be used to evaluate apical leakage, were embedded in acrylic blocks with apical 2 mm sections exposed. The exposed root surface was covered with a nail polish to leave a 1 mm apical opening. In samples that should be evaluated for coronal leakage, the apical part was embedded and the exposed part of the coronal region was covered. Unlike those in the negative control groups, the area where the leakage was to be evaluated was completely covered with nail polish.

All samples were incubated for 72 h in the dye solution at 37 °C, with 2% methylene blue (Kim San Medicine, İstanbul, Turkey). After 72 h, the samples were washed with tap water for 5 minute and dried. The specimens were divided into two with a water-cooled diamond disc in the bucco-lingual direction (Moddental Medical Testing Devices, Ankara, Turkey).

The prepared sections were examined with X150 magnification under a stereomicroscope (Olympus SZ61, Tokyo, Japan). The amount of progress in the dye was measured micrometrically using the Olympus Stream Image Analysis Software (Olympus, Tokyo, Japan) program. The highest leakage value observed in each sample was considered. Images and numerical values transferred from the microscope to the computer were recorded.

The SEM study for the evaluation of dentin adaptation was conducted at the Erciyes University Technology Research and Application Centre laboratory. A total of 16 teeth were prepared with four canals filled with each material. The investigations were conducted under the ×1000 magnification with

Leo 440 computer-controlled Digital SEM (Zeiss, Oberkochen, Germany). Using the Image-Pro Plus Software program, the distance between the dentin and the material was measured at four points, and the average of 16 values obtained from each group was calculated. The results were evaluated statistically.

Statistical Analysis

All statistical analyses were performed using the "Statistical Package for the Social Sciences" (SPSS 24, SPSS Inc., Chicago, Illinois, USA). The Shapiro-Wilk and Kolmogorov-Smirnov tests were used to determine the normal distribution fit of the data and the homogeneity of the variances. The Mann-Whitney U test was used to compare the difference between the two independent samples according to the normality results of the data; the Kruskal-Wallis test and ANOVA tests were used to compare the differences between the two groups. The level of significance in tests was <0.05. The two groups had statistically significant differences according to the level of significance.

Results

The average microleakage values of the experimental and control groups are shown in Table 2.

Statistical analysis revealed that the Biodentine group in the apical and coronal regions showed more microleakage at the statistically significant level than the Angelus MTA and Retro MTA group. The difference between the Ortho MTA group and all other groups was not statistically significant, those between Angelus MTA and Retro MTA were significant (p>0.05).

When examining the relationship between coronal and apical microleakage values of the same material, no statistically significant difference was observed between the two regions in all materials (p>0.05).

The mean values obtained from the measurements from the SEM images and between groups distributions are shown in Table 3. The difference between the groups in the apical region was not statistically significant. A statistical difference was found between Biodentine and other groups in the coronal region (p<0.05). No statistically significant difference was found between the MTA groups (p>0.05). The images examined are shown in Figures 1-4.

Discussion

In young permanent teeth that differ from teeth that have developed morphologically, leakage after endodontic treatment negatively affects the prognosis.

MTA is widely used in single visit apexification treatments; however, new materials are being developed to eliminate its disadvantages. The easiest is the Angelus MTA, which shortened the setting time. *In vitro* conditions, a single visit apexification applied in the teeth has been successful in preventing microleakage and promoting fracture resistance (6,7). Moore et al. (8) found that Angelus MTA was successful in a single visit apexification treatment in an average

Table 2. Linear microleakage values of the experimental and control groups (µm)

Group		N	Mean	Median	SD
Angelus MTA	Apical	20	1833.50*	1747.50	804.15
	Coronal	20	1800.15*	1731.00	847.85
Ortho MTA	Apical	20	2109.55*†	1686.50	1469.86
	Coronal	20	2087.80*†	2061.50	907.53
Retro MTA	Apical	20	1829.00*	1787.00	689.34
	Coronal	20	1647.25*	1263.00	1221.38
Bio dentine	Apical	20	4018.95†	2890.00	3098.25
	Coronal	20	3347.85†	2442.00	2505.03
Positive control	4	Apical	12000.00	12000.00	0.00
	4	Coronal			
Negative control	Apical	8	0.00	0.00	0.00
	Coronal	8	0.00	0.00	0.00

*Statistically significant values in the same column were shown with different symbols (p<0.05). MTA: Mineral trioxide aggregate, SD: Standard deviation

Table 3. SEM dentin-material spacing measurement averages and inter-group distribution

Group		N	Mean	SD
Angelus MTA	Apical	2	2.84*	3.92
	Coronal	2	2.54*	4.05
Orhto MTA	Apical	2	2.13*	3.10
	Coronal	2	2.30*	2.79
Retro MTA	Apical	2	2.63*	4.07
	Coronal	2	2.66*	3.77
Biodentine	Apical	2	4.38*	3.02
	Coronal	2	5.18†	1.31

*Statistically significant values in the same column are shown with different symbols (p<0.05). MTA: Mineral trioxide aggregate, SD: Standard deviation

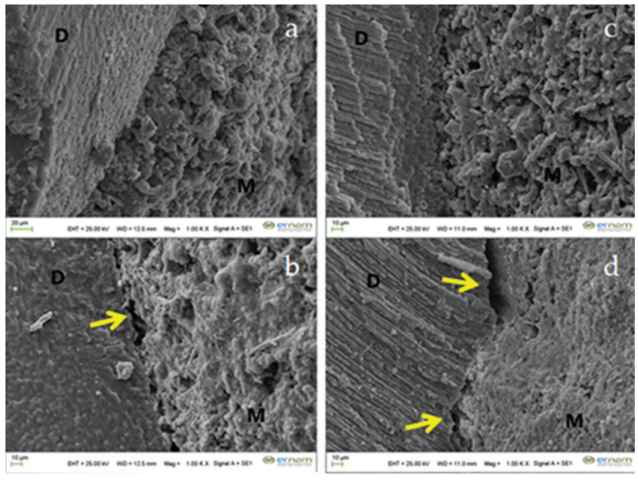

Figure 1. Images of the samples filled with Angelus MTA. a.b. Apical region images c.d. Coronal region images
MTA: Mineral trioxide aggregate, D: Dentin, M: Material (The spaces between the material and dentin are indicated by arrows.)

of 23.4 months following the clinical trial. Ortho MTA, which has been recently developed, has been shown to be an alternative to conventional canal treatment methods after the microleakage assessment of single rooted permanent teeth (9). It was also found to be marginally adaptive in retrograde cavities (10). Retro MTA, defined as zirconium oxide-containing calcium aluminate, is in the foreground for short setting time (11). Biodentine appears to be an alternative to single visit apexification treatments with short setting time and no discoloration. Biodentine has also been reported to show low microleakage and high dentin adaptation. It has been shown as a successful material

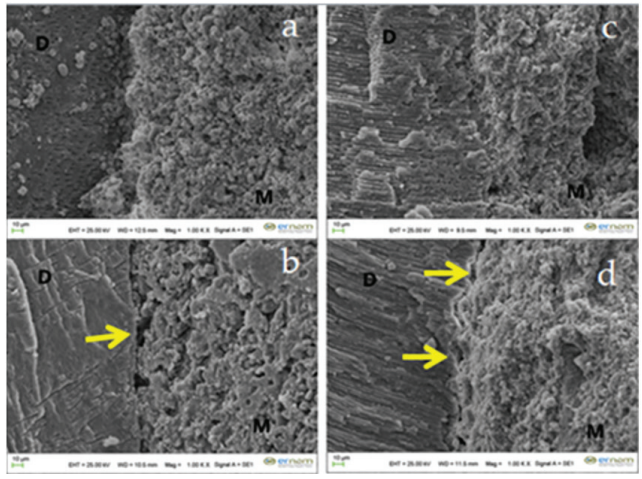

Figure 2. Images of the samples filled with Ortho MTA. a.b. Apical region images c.d. Coronal region images
MTA: Mineral trioxide aggregate, D: Dentin, M: Material (The spaces between the material and dentin are indicated by arrows.)

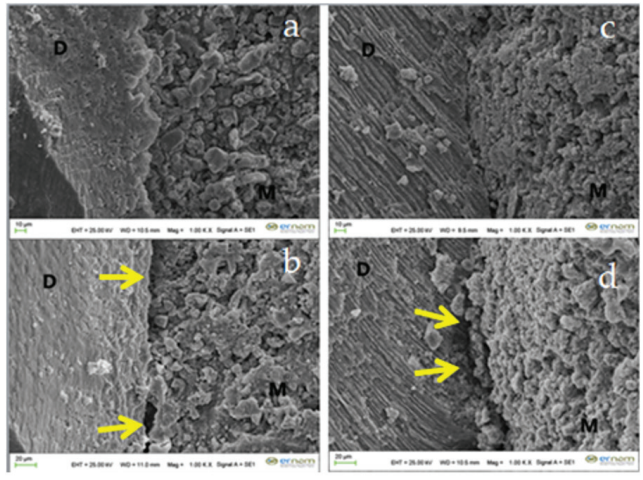

Figure 3. Images of the samples filled with retro MTA. a.b. Apical region images c.d. Coronal region images
MTA: Mineral trioxide aggregate, D: Dentin, M: Material (The spaces between the material and dentin are indicated by arrows.)

in single session apexification treatments in many case reports (12-14).

Nur et al. (15) evaluated the microleakage using dye penetration method, MTA had lower leakage values compared to Biodentine. These findings are consistent with the results of our study. No statistically significant difference was found between Biodentine and MTA in other microleakage studies in which teeth with open apex roots were replicated (16,17). It was thought that the difference between the MTA and

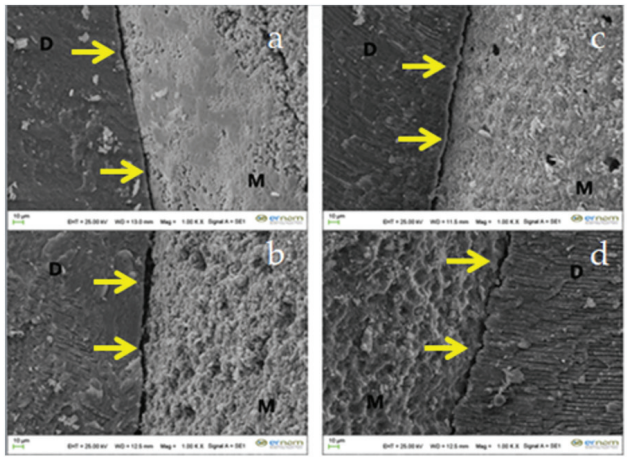

Figure 4. Images of the samples filled with Biodentine. a.b. Apical region images c.d. Coronal region images
MTA: Mineral trioxide aggregate, D: Dentin, M: Material (The spaces between the material and dentin are indicated by arrows.)

microleakage evaluation method used in our study from other studies may be related to the results.

In order to prevent failures in endodontic treatments, not only apical but also coronal leakage should be tested (18,19). Therefore, coronal leakage evaluation was performed in our study. The lowest coronal leakage according to the obtained data was shown by Retro MTA, followed by Angelus MTA and Ortho MTA. The largest coronal microleakage value was observed in the Biodentine group. Statistically, Biodentine showed more leakage at a significantly higher level than the Angelus and Retro MTA groups, but no significant difference was found among the other groups.

The relationship between microleakage and clinical success of the root canal system is controversial. Some researchers argue that clinical success rates do not correlate with the microleakage values and that these studies may provide questionable results without assessing the quality of care (20,21). On the contrary, studies that evaluate microleakage in the root canal system continued. Some authors refer to the consideration of treatment methods and criteria in understanding the characteristics and mechanism of the materials (5,22). More *in vitro* and long-term clinical trials are needed to compare the extent of microleakage and clinical success.

SEM plays an important role in assessing the marginal compatibility of materials used in the root canal system, the adaptation of apical barriers in the incomplete development of teeth, and the microstructure of materials. Marginal adaptation correlates with the sealing ability of dental materials and therefore influences the clinical success rate. Brenes-Valverde et al. (17) underwent a single visit apexification treatment to provide a 4 mm apical plug with Biodentine and MTA on the teeth with open apex form. According to their result, the two materials showed similar success rates in marginal adaptation. In our study, although Biodentine had a higher mean of gap size between dentin-material than MTA groups, the difference between them was not statistically significant.

Other materials for microleakage and marginal adaptation showed more successful results than Biodentine. This condition may be due to the possible manipulation of Biodentine in terms of placement in the root canal system via the orthograde and the consideration of short setting time. The other materials did not show a significant difference between their results, because they exhibited more similar characteristics in terms of structure.

Conclusion

The microleakage data and SEM evaluation results obtained in this study are parallel and support each other. Biodentine gives more negative results in both evaluations compared to other groups. Other materials did not show a significant difference in their results. However, *in vitro* and *in vivo* studies are needed to evaluate the use of these biomaterials in apexification therapies.

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Ethics

Ethics Committee Approval: This study was approved by the Aydın Adnan Menderes University Medical Faculty Non-Invasive Clinical Research Ethics Committee on 14 June 2016 with the decision number 25.

Informed Consent: Informed consent is not required.

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Authorship Contributions

Surgical and Medical Practices: M.D., K.G.U.G., Concept: K.G.U.G., Design: M.D., K.G.U.G., Data Collection or Processing: M.D., K.G.U.G., Analysis or Interpretation: M.D., K.G.U.G., Literature Search: M.D., K.G.U.G., Writing: M.D., K.G.U.G.

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