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Original Research Article

Evaluation of Marginal Adaptation of Three Biomaterials as Apical Barrier in Experimental Apexification Model

Deneysel Apeksifikasyon Modelinde Apikal Bariyer Olarak Üç Farklı Biyomateryalin Marjinal Adaptasyonunun Değerlendirilmesi

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ABSTRACT

Aim: The purpose of this study was to compare the marginal adaptation of Mineral Trioxide Aggregate (MTA), Biodentine, and EndoSequence as apical plug materials after orthograde placement in an experimental apexification model using confocal laser scanning microscopy (CLSM).

Materials and Method: The study was conducted with sixty single-rooted mature mandibular premolar teeth. These teeth were prepared as 12 mm root lengths, and apical regions of teeth were enlarged by using peeso reamers to give the open apex form likely with the immature teeth as an experimental apexification model. ProRoot MTA, Biodentine, and EndoSequence used as apical plug materials were placed into the apical region of samples with a thickness of 4 mm. After filling the remaining root canal with gutta-percha, each sample was divided into three transverse sections by using a hard tissue microtome to evaluate marginal adaptation between dentine walls and apical plug material using a confocal laser scanning microscopy. Then, gap areas of all microscope images were measured with Image J program.

Results: MTA showed the highest marginal adaptation, followed by EndoSequence and Biodentine. Biodentine was significantly inferior to the other materials in marginal adaptation ($p<0.01$). There were no significant differences between EndoSequence and ProRoot MTA groups ($p>0.01$).

Conclusion: MTA and EndoSequence exhibited superior marginal adaptation as apical plugs compared to Biodentine. Biodentine had the poorest adaptation among the three materials.

Keywords: Apexification; Apical plug; Calcium silicate-based cements; Confocal laser scanning microscopy; Immature permanent teeth

ÖZET

Amaç: Bu çalışmanın amacı, deneysel apeksifikasyon modelinde ortograt yerleştirme sonrasında Mineral Trioksit Agregat (MTA), Biodentin ve EndoSequence'in apikal bariyer materyalleri olarak marjinal uyumunun, konfokal lazer tarama mikroskop kullanarak karşılaştırmaktı.

Gereç ve Yöntem: Çalışmada, 60 adet matür tek köklü mandibular premolar diş kullanıldı. Dişler 12 mm kök uzunlukları olacak şekilde hazırlandı ve dişlerin apikal bölgesi immatür dişlerdeki açık apeks formunu vermek için peeso reamer kullanılarak genişletildi. Apikal bariyer materyali olarak kullanılan ProRoot MTA, Biodentin ve EndoSequence, örneklerin apikal bölgesine 4 mm kalınlığında yerleştirildi. Kalan kök kanalı gutta-perka ile doldurulduktan sonra her örnek, marjinal adaptasyonu değerlendirmek için sert doku mikrotomu kullanılarak üç enine kesite bölündü. Lazer taramalı konfokal mikroskobu kullanılarak dentin duvarları ve apikal bariyer materyali arasındaki marjinal adaptasyon değerlendirildi. Daha sonra tüm mikroskop görüntülerinin boşluk alanları Image J programı ile ölçüldü.

Bulgular: MTA en yüksek marjinal uyumu gösterirken, onu EndoSequence ve Biodentine izlemiştir. Biodentinin marjinal adaptasyonu diğer materyallere kıyasla anlamlı derecede daha düşük olduğu bulunmuştur ($p<0.01$). EndoSequence ve ProRoot MTA grupları arasında anlamlı bir fark bulunmamıştır ($p>0.01$).

Sonuç: MTA ve EndoSequence materyalleri Biodentine kıyasla apikal bariyer olarak üstün marjinal adaptasyon sergilemiştir. Üç materyal arasında en zayıf marjinal adaptasyonu Biodentin göstermiştir.

Anahtar Kelimeler: Apeksifikasyon; Apikal bariyer; Kalsiyum silikat bazlı simanlar; Lazer taramalı konfokal miroskop; İmmatür daimi dişler

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INTRODUCTION

Apexification is the most common treatment method for immature permanent teeth that induce the physiologic formation of a hard tissue barrier to achieving apical closure.[1,2] Calcium hydroxide apexification is the most used traditional method that requires prolonged root canal dressings to create an apical barrier. However, the disadvantages of this treatment are an increased risk of tooth fracture and interruption of treatment.[3]

Advancements in biomaterial sciences have significantly enhanced the effectiveness and practicality of apexification procedures. Mineral trioxide aggregate (MTA) has been widely accepted as the preferred material for single-visit apexification due to its effective performance as an apical plug.[4-6] Current studies[7-9] in the endodontic literature have mainly focused on using MTA as an apical plug. Despite its popularity, certain challenges associated with the use of ProRoot MTA (Dentsply Tulsa Dental, Tulsa, USA), such as its high expense, difficulty in handling, and prolonged setting duration, have been identified.[10] To address these challenges, new calcium silicate-based formulations like Biodentine (Septodont, France) and EndoSequence BC Root Repair Material (Brasseler, USA) have been developed. These materials offer similar biological effects and essential components to MTA.[11]

Biodentine is available as a capsule form, containing a premeasured amount of calcium chloride solution and a separate powder, which is activated by mechanically mixing in an amalgamator. The setting time of biodentine is approximately 12 minutes. It has high mechanical performance and excellent biocompatibility.[5,12,13] The mechanical properties of Biodentine are higher than those of MTA and similar to dentine.[5]

EndoSequence is premixed and produced as a ready-to-use putty or injectable form with easier handling and application.[5,14] The manufacturer's specifications indicate that the inherent moisture within the dental tubules is adequate for the setting process. EndoSequence BC RRM-Fast Set Putty (Brasseler, USA), with new fast set chemistry, has a setting time of 20 minutes. Its nanosphere-sized particles are engineered to penetrate the dental tubules, leveraging the intrinsic fluid of the dentine to establish a micromechanical bond upon hardening.[12]

Prior studies examining the effectiveness of these biomaterials through orthograde filling of immature teeth utilizing confocal laser scanning microscopy are nonexistent. This study aims to assess the marginal adaptations of MTA, Biodentine, and EndoSequence as apical plug materials by using a confocal laser scanning microscope in an experimental apexification model. The null hypothesis was no significant difference in marginal adaptation among the various calcium silicate-based cements when used as apical plug materials in apexification treatments.

MATERIALS AND METHOD

This study was approved by the Ethics Committee of Clinical Research of Gazi University Faculty of Dentistry (number: GÜDHKAEK.19.05/4). Single-rooted mandibular premolar teeth were selected for the present study. The teeth were examined under a microscope (Carl Zeiss OPMI Proergo; Carl Zeiss Meditec AG, Jena, Germany) to exclude teeth with carious lesions, cracks, fracture lines, or resorption. Roots were debrided to eliminate soft tissue, subsequently disinfected with 5.25% sodium hypochlorite (NaOCl) for one hour and preserved in saline until experimentation commenced.

Sample Preparation

To standardize root length at 12 mm, the crowns were removed using a diamond disc (Sunshine Diamond, Germany) with water cooling. Root canals were accessed, and the working length was determined with a #15 K-file. Canal preparation followed, scaling up to a #40 master apical file via K-files, subsequently expanded to #80 using the step-back method. The root canals were irrigated with 2 mL 5.25% NaOCl (Endosolv-HPV, Imicryl, Türkiye) during instrumentation. For mimicking immature teeth with open apices, the apical foramen was enlarged to a diameter of 1.70 mm using progressive sizes (1-6) of Peeso Reamers (Mani, Inc., Tochigi, Japan). The smear layer was removed with 17% Ethylenediamine Tetra Acetic Acid (EDTA) (DiaPrep Plus, DiaDent, South Korea) irrigation, and a final rinse with saline was performed. The samples were stored in sterile saline until further processing.

Experimental Procedure

The samples were randomly divided into three groups, each assessing a different apical plug material (n=20 per group):

Group 1: MTA (ProRoot MTA; Dentsply Tulsa Dental, Tulsa, USA)
Group 2: Biodentine (Septodont, Saint Maur des Faussés, France)
Group 3: EndoSequence (EndoSequence BC RRM-Fast Set Putty, Brasseler, USA)

The samples were stabilized in a moistened floral arrangement substance (Oasis Floral Foam, Kent, USA) to simulate periapical tissues *in vivo* conditions. The root canals were dried with paper points (Dentplus, DiaDent, Netherlands), apical plug materials were prepared according to the manufacturer's instructions, and placed into the canals in the orthograde direction, compacted with endodontic pluggers (Kerr Co., USA). Periapical radiographs were taken to confirm the density and thickness of the apical plug. A moist cotton pellet was applied proximal to the apical plug, and a temporary sealant (Coltosol F, Coltene Whaledent, Langenau, Germany) was applied. Samples were incubated at 37°C with 100% humidity to allow for material setting, as per manufacturer's guidelines. The canal space above the set apical plug was filled with gutta-percha (DiaDent, Almere, The Netherlands) and AH-PlusTM Sealer (Dentsply, Maillefer, Netherlands), using the lateral compaction technique. The root canal orifices were then sealed with a composite material (Filtek Supreme XTE, 3M ESPE), and samples were again stored under the same conditions for one week to ensure sealer setting.

Confocal Laser Scanning Microscope Evaluation

Three coats of nail varnish were applied to the external root surfaces of the samples, excluding the apical 1.0 mm, to prevent any leakage from the lateral canals or dentinal tubules. The samples were then immersed in Rhodamine B (Merck, Turkey) for 48 hours and cleansed to remove excess dye for 15 minutes. The samples were cut into three sections in the transverse direction with 1 mm thickness, using hard tissue microtome (ATM Brilliant 210 Cutter, ATM GmbH, Mammelzen, Germany) and 0.3 mm microtome saw (Isomet Buchler, Lake Bluff, IL) (Figure 1). The marginal adaptation between dentin walls and apical plug material for all sections was examined under a confocal laser scanning microscope (CLSM; Zeiss Lsm 510 Meta, Zeiss GmbH, Germany) at 20x magnification. The images obtained were analyzed using Image J software (National Institutes of Health, Bethesda, Maryland, USA) to calculate the gap areas. A single operator executed all measurements thrice to ensure reliability. The mean gap area for each sample was calculated by averaging all gap areas from every section in each root.

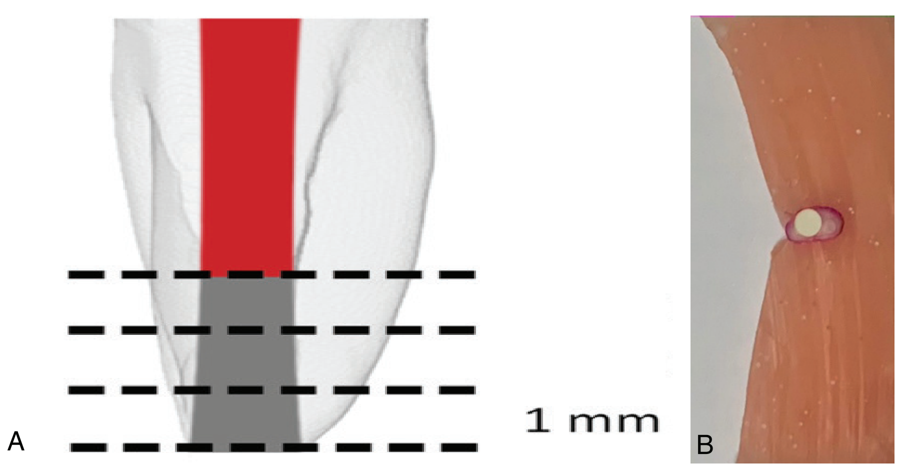

Figure 1. A) Schematic representation of the methodology used in this study.
B) Representative photograph of 1 mm sections obtained with a microtom

Statistical Analysis

SPSS for Windows version 23.0 was used for statistical analysis. The minimum sample size was determined to be n=20 for each of the three groups based on a 15% mean difference in outcome measurement in at least one the pairwise comparisons, statistical power = 0.85, and statistical significance (alpha) level = 0.01. Shapiro-Wilk test was used to determine whether the measurements were compatible with normal distribution. One-Way ANOVA test was used to compare measurements according to groups. Tukey's post-hoc HSD test was used to assess the statistical significance of pairwise comparisons once the statistically significant ANOVA test results was obtained. Significance was evaluated at the level of $p<0.01$.

RESULTS

The CLSM examination of these transverse sections showed marginal gaps between the dentine and apical plug material interface. Figure 2 shows CLSM images of MTA, Biodentine, and EndoSequence material under 20X, respectively. Shapiro-Wilk test indicated that the data was normally distributed ($p>0.01$). The gap area between the dentinal walls and material used was compared using One Way ANOVA, which elucidated a statistically significant difference amongst the three groups ($p<0.01$). The mean value of the gap area and standard deviations for each group are shown in Table 1.

When the materials were evaluated as pairs, there were significant differences between EndoSequence and Biodentine and MTA and Biodentine ($p<0.01$). Contrastive analysis between the groups indicated a lack of significant difference between the EndoSequence and MTA groups ($p>0.01$), while each showed significant differences when compared with Biodentine ($p<0.01$).

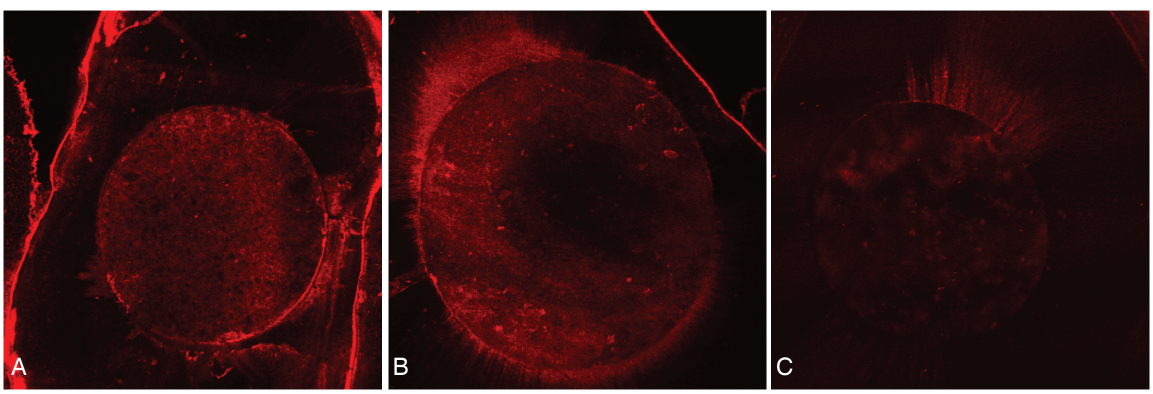

Figure 2: Representative confocal images (X20) of different groups a) MTA, b) Biodentine, c) EndoSequence

Table 1. Minimum, maximum, and mean marginal gap area (μm2) and standard deviation seen under CLSM (20X)

	Minimum gap area	Maximum gap area	Mean value	Standard deviation	P-Value
MTA	148356	499162	220814	94614	
Biodentine	426178	1335699	743937*	230810	p<0.01
EndoSequence	30643	756164	379326	218015	

*: p<0.01

DISCUSSION

The effects of the various calcium silicate-based cements when used as apical plug materials in apexification treatments on the marginal adaptation were investigated in this *in vitro* study. The results showed that the adaptation of MTA and EndoSequence were higher than Biodentine thus, the null hypothesis that the marginal adaptation would not be significantly affected by the different apical plug materials was partially rejected

Calcium silicate-based biomaterials have emerged as a prominent choice in the dental field, necessitating a comprehensive examination of their efficacy across varied clinical scenarios. These materials are routinely used for various purposes, including apical plug materials during the apexification procedure.[2,15] MTA has been preferred in recent years in apexification procedures due to its biocompatibility and physical and chemical properties, and recent studies have reported successful long-term clinical outcomes.[6,7,9] However, despite its advantageous aspects, certain limitations of MTA have been documented, notably its extended setting duration, challenges associated with clinical application, poor handling properties, and high cost. [5,15]

Commercially available calcium silicate-based materials, such as Biodentin and EndoSequence, possess improved handling characteristics and color stability while maintaining a suite of physical and chemical characteristics that align well with those of MTA.[15] Research has predominantly focused on these substances' physical properties, particularly their efficacy in sealing and marginal adaptation as retrograde root-end-filling material during surgical procedures.[4,14-19] However, limited studies compared the marginal adaptation of MTA with these materials for orthograde obturation of immature teeth, which is generally used by pediatric dentistry for apexification.[2,20,21] The endodontic literature mainly focuses on MTA's use as an apical plug, and high success rates and good sealing ability of MTA reported.[6,8,9,18,19] In this study, MTA showed the highest marginal adaptation compared to other apical plug materials. Excellent adaptation to the dentine from the expansion of the material during the hydration reaction has been reported in the studies evaluating marginal adaptation.[22]

This study indicated no significant difference between the marginal adaptation of MTA and EndoSequence. This result is consistent with the results of the four recent studies.[2,13,15] They compared the marginal adaptation of MTA and EndoSequence with different methods, and all of them showed that these two materials had similar marginal adaptation. Endosequence's nanosphere particle size allows the material to enter the dentinal tubules. When the material hardens, it forms a mechanical bond with dentin and creates dimensional stability by limiting the shrinkage potential of the material.[5] The successful adaptation of EndoSequence is thought to depend on this.

According to this study, MTA and EndoSequence were statistically higher than Biodentine. Biodentine had the poorest adaptation among the three materials. The findings herein present a contrast to prior research which assessed apical leakage between MTA and Biodentine.[4,17,18,20,21,23] These studies found that Biodentine showed superior sealing ability compared to MTA. Until this point, the literature lacks a study that specifically evaluates the marginal gap of MTA, Biodentine, and EndoSequence when used as an orthograde apical barrier utilizing the CLSM technique. It is plausible that the methodological approach employed in the current study has contributed to the variance observed in outcomes.

The thickness of the apical barrier plays a significant role in maintaining an adequate seal. Several studies have been conducted to show sufficient thickness when used to create an apical barrier. They have reported that the ideal thickness for routine clinical use is 4 mm.[4,24] Therefore, in our study, apical plug materials were used with a thickness of 4mm.

The present study assessed the marginal adaptation between dentin and apical plug material using a confocal laser scanning microscope. While CLSM has been employed in a myriad of studies to examine the interface integrity of silicate-based materials, these investigations have predominantly focused on the quality of retrograde filling with various calcium silicate-based substances.[16,19,25,26] However, these studies evaluated the retrograde filling quality in different calcium silicate-based materials. None of them were performed on orthograde placement. The technique of orthograde insertion, as utilized for

MTA, EndoSequence, and Biodentine in this study, demands greater precision due to the challenges inherent in condensing materials where resistance is minimal owing to the open apex's nature. Furthermore, the intricacies involved in placing materials within the apical area, alongside anatomical variations, can complicate achieving a seamless adaptation to the dentinal walls, potentially leading to the formation of marginal gaps at the interface between the dentin and the material.[22]

CLSM has several advantages, namely minimal specimen preparation, and the same sample can be used for both treatment and further processing, the acquisition of high-resolution images, and the ability for immediate observation. At the same time, many different components can be examined using various dyes. Within the scope of this research, Rhodamine B, a dye that emits fluorescence under the stimulation of red light at a wavelength of 546 nm, was employed.[19,27] In this study, MTA, Biodentine, and EndoSequence showed sensitivity to dye uptake, and different rates of dye absorption were observed in the materials. In previous studies[16,27], where Rhodamine B was used to measure marginal adaptation of MTA, it was reported that the material absorbs Rhodamine B instead of advancing at the dentin-material interface. Conversely, another study did not observe any alteration in the material upon interaction with the dye.[28]

In this study, the experimental apexification model was adapted from previous studies.[29,30] Although individual differences are always possible between different teeth, great care was taken in selecting the experimental teeth. Constructing *in vitro* immature teeth with an open apex model is challenging because it is difficult to replicate the anatomical relationship between the open apex and periodontal tissue. Future studies are needed to confirm this study's results and further investigate the behavior of these materials *in vivo* conditions.

CONCLUSION

This study indicated no significant difference between the marginal adaptation of MTA and EndoSequence. Biodentine had the poorest adaptation among the three materials. EndoSequence BC Root Repair Material is a promising material with high marginal adaptability that overcomes the limitations of MTA.

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

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