Original Research Article

Evaluation of Dentinal Tubule Penetration of NeoMTA Plus in the Presence of EDTA and Etidronic Acid

EDTA ve Etidronik Asit Varlığında NeoMTA Plus'ın Dentin Tübül Penetrasyonunun Değerlendirilmesi

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ABSTRACT

Aim: The aim of this study was to evaluate the dentinal tubule penetration of NeoMTA Plus and AH Plus root canal sealers in root canals applied with etidronic acid (HEBP) and Ethylene diamine tetra acetic acid (EDTA) by using Confocal Laser Scanning Microscopy (CLSM).

Material and Methods: The study was performed on 102 extracted human mandibular premolar teeth with single canals. Chemomechanical preparation was done using Ni-Ti rotary files with 2% NaOCl irrigation. The teeth in the study group were evaluated in terms of chelation agents and root canal sealers in six experimental groups: Group 1:HEBP-AH Plus, Group 2:HEBP-NeoMTA Plus, Group 3:EDTA-AH Plus, Group 4:EDTA-NeoMTA Plus, Group 5:Saline-AH Plus, Group 6:Saline-NeoMTA Plus. After the final irrigation, the root canals were obturated with NeoMTA Plus and AH Plus which were mixed with 0.1% Rhodamin B dye. Teeth were sectioned at 1 mm and 5 mm from the apex. The maximum tubule penetration and percentage of penetration values were obtained from the CLSM images and were statistically analyzed using a two-way analysis of variance (ANOVA) with Bonferroni correction ($p < 0.05$).

Results: In the penetration depth analysis at 1 mm, there was a statistically significant difference between Group 1 and Group 5 ($p = 0.017$). In 5 mm, Group 5 showed a lower penetration depth than Group 1 and Group 3 ($p = 0.005$). In 1 mm, Group 5 showed a lower penetration percentage than Group 6 and Group 3 ($p=0.030$).

Conclusions: The NeoMTA Plus showed better tubule penetration results than the AH Plus and removal of the smear layer increased dentin tubule penetration.

Keywords: CLSM; dentinal tubule penetration; EDTA; etidronic acid; NeoMTA Plus

ÖZET

Amaç: Bu çalışmanın amacı EDTA ve etidronik asit solüsyonları uygulanan kök kanallarında NeoMTA Plus® ve AH Plus® kök kanal patlarının dentin tübül penetrasyonunu konfokal lazer taramalı mikroskop ile incelenmesidir.

Gereç ve Yöntem: Çalışmada 102 adet tek köklü, kök gelişimini tamamlamış, insan alt çene premolar dişleri kullanıldı. Kemomekanik preparasyon, 10 ml %2 NaOCl irrigasyonu kullanılarak Ni-Ti döner eğe sistemleri ile yapıldı. Dişler kullanılacak şelasyon ajanları ve kök kanal patlarına göre, her biri 17 örnekten oluşan altı deney grubuna ayrıldı: Grup 1: HEBP-AH Plus®, Grup 2: HEBP-NeoMTA Plus®, Grup 3: EDTA-AH Plus®, Grup 4: EDTA-NeoMTA Plus®, Grup 5: Salin-AH Plus®, Grup 6: Salin-NeoMTA Plus®. Final irrigasyonunun ardından kök kanalları Rhodamin B ile muamele edilmiş NeoMTA Plus® ve AH Plus® kök kanal patları ile tek kon tekniği kullanılarak dolduruldu. Obturasyon işlemlerinden sonra apeksten 1 ve 5 mm mesafeden horizontal yönde kesitler alınıp konfokal lazer taramalı mikroskop ile incelendi. Maksimum tübül penetrasyonu ve penetrasyon yüzdesi değerleri Konfokal lazer mikroskopi (CLSM) görüntülerinden elde edildi. İkili karşılaştırmalarda Bonferroni düzeltmesi ile iki yönlü varyans analizi (ANOVA) kullanılarak istatistiksel olarak analiz edildi ($p < 0.05$).

Bulgular: 1 mm'deki penetrasyon derinliği analizinde Grup 1 ve Grup 5 arasında istatistiksel olarak anlamlı fark vardır ($p=0.017$). 5 mm'de Grup 5, Grup 1 ve Grup 3'e göre daha düşük penetrasyon derinliği göstermiştir ($p=0.005$). 1 mm'de Grup 5, Grup 6 ve Grup 3'ten daha düşük penetrasyon yüzdesi göstermiştir ($p=0.030$).

Sonuç: Smear tabakasının kaldırılması kök kanal patlarının dentin tübül penetrasyonunu artırmıştır. NeoMTA Plus® kök kanal patı AH Plus®'tan daha yüksek tübül penetrasyon değerleri göstermiştir ve smear tabakasının kaldırılması dentin tübül penetrasyonunu artırmıştır.

Anahtar kelimeler: CLSM; dentin tübül penetrasyonu; EDTA; etidronik asit; NeoMTA Plus

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INTRODUCTION

The success of the root canal treatment is based on mechanical preparation of the root canal system, disinfection with irrigation solutions, and 3-dimensional obturation from the coronal to the apical.[1]

The penetration of the root canal filling material into the dentine tubules is associated with the elimination of the smear layer during the preparation processes. The smear layer creates a physical barrier, inhibiting the penetration and adhesion of root canal sealers into the dentine tubules.[2]

Various chelating agents have been proposed to remove the smear layer. Traditionally, sodium hypochlorite (NaOCl) and ethylene diamine tetra acetic acid (EDTA) are used to eliminate the smear layer. However, EDTA's interactions with NaOCl reducing the latter's antibacterial and tissue solvent property, its inadequacy to remove the smear layer from apical thirds of the root canal, and erosion created in the root dentine when it is applied for more than 1 min, have resulted in the search for alternative chelation agents rather than EDTA.[3]

Etidronic acid [1-hydroxyethylidene-1,1-bisphosphonate (HEBP)], is a non-toxic, weakly acidic bisphosphonate that is less aggressive on root dentine while removing the smear layer.[4] It was suggested to be a chelating agent that can be used together without affecting the antimicrobial properties of NaOCl.[5]

Recently, calcium silicate-based materials have been produced with good biocompatibility and bioactivity. One of these sealers, NeoMTA Plus (Avalon Biomed, Bradenton, FL), is a new fine powder tricalcium silicate material mixed with a water-based gel that provides easy handling properties and contains tantalum oxide as a radiopacifying agent.[6] Tricalcium silicate-based materials induce biomineralization by interaction with dentine fluid and the creation of mineral plugs within the dentine tubules, thereby increasing biological activity and root canal sealing within the root canal.[7]

Confocal Laser Scanning Microscope (CLSM) is one of the imaging methods used to evaluate the penetration ability of root canal sealers. CLSM is the most effective method due to its advantages in analyzing larger areas with small magnifications, examining with high-resolution images, and it could provide a detailed view of the presence and distribution of root canal sealers inside dentine tubules when the fluorescent dye was added into the sealers.[8] In addition to CLSM does not require any special specimen processing that may produce artifacts, and the observations can be made under normal conditions.[9]

The residues of irrigation solution remain on the root canal wall after irrigation are in contact with root canal sealers for a long time. For this reason, it is important to investigate and understand the interaction between these irrigation solutions and tricalcium silicate-based materials.[10]

Previous studies showed that the use of acids such as EDTA impairs gel formation of tricalcium silicate-based materials, which reduces the micro-hardness of the material as well as its compressive strength.[10] When calcium silicate cement is used, it is recommended to use weaker acids to remove the smear layer.[11]

Studies evaluated the impacts of etidronic acid on the smear layer[4,12] , however, no study has been made of the effect of tricalcium silicate cement on tubule penetration of dentine. Therefore, the aim of this *in vitro* study was to evaluate the dentinal tubule penetration of a new root canal sealer, tricalcium silicate-based NeoMTA Plus, in the presence of etidronic acid, EDTA and saline solutions compared to AH Plus by using a Confocal Laser Scanning Microscopy.

MATERIAL AND METHODS

This study was approved by the Gazi University Faculty of Dentistry Clinical Research Ethics Committee (06.12.2018- E.45576) and it became performed according to the Declaration of Helsinki. In this study, one hundred two human mandibular premolar teeth with single canal and single apical foramen, complete apical development, curvature ratio of fewer than 10 degrees, and extracted from periodontal or orthodontic reasons were selected. Periapical radiographs have been taken from the mesiodistal and buccolingual directions to verify the presence of a single canal. Teeth were examined under a stereomicroscope, teeth with fractures, cracks, and defects were excluded from the study. The 10-K file was used to check whether the apical

foramen of the roots was open. The teeth were stored in 5.25% NaOCl solution during a day to remove the organic residues on the root surfaces. The outer root surface of each tooth was cleaned by scraping it with periodontal currete (#3-4 Gracey, Nordent, USA). Teeth were kept in 0.9% saline at 5 °C before the experiment.

Teeth were decoronated under water spray to provide a standard root length of 12±1 mm. The working length was determined by subtracting 1 mm from the distance to the apical foramen. Root canals were prepared using ProTaper Universal rotary instruments (Dentsply Maillefer, Ballaigues, Switzerland) up to size F4 (40/.06). During instrumentation, the canals were irrigated with 2 mL of 2% NaOCl solution (Imicryl , Konya, Turkey) at each instrument change.

The specimens were randomly divided into six groups, according to the chelation agents and sealer type (n=17).

Group 1 (n=17): HEBP-AH Plus.
Group 2 (n=17): HEBP-NeoMTA Plus.
Group 3 (n=17): EDTA-AH Plus.
Group 4 (n=17): EDTA-NeoMTA Plus.
Group 5 (n=17): Saline-AH Plus.
Group 6 (n=17): Saline-NeoMTA Plus.

After instrumentation, the canals in group1 and group 2 were irrigated with 2.5 mL 9% etidronic acid (Sigma-Aldrich, St. Louis, MO, ABD), group 3 and group 4 were irrigated with 2.5 mL %17 EDTA (Werax, İzmir, Turkey) and group 5 and group 6 were irrigated with 2.5 mL saline solution. The irrigation solutions using a 27-G side vented needle (Ultradent Products Inc., South Jordan, UT, USA), which was placed 1 mm short of the apex, were applied for 1 min, without any activation.

After the irrigating procedures, canals were rinsed with 5 mL of distilled water and then dried using paper points (DiaDent, Korea). AH Plus (Dentsply Maillefer, Ballaigues, Switzerland) and NeoMTA Plus were mixed according to the manufacturer's instructions. Root canal sealers were labeled with 0.1% Rhodamine B (Sigma-Aldrich, St. Louis, MO, ABD) in order to be visualized under a confocal laser scanning microscope. All root canal sealers were placed into the canal using a size 30 Lentulo spiral where 1-mm short of the working length. A single gutta-percha cone (ProTaper Universal F4, Dentsply Maillefer) was then lightly coated with the AH Plus and NeoMTA Plus sealers for the respective cases and placed in the root canal to the working length. The coronal opening was filled with a temporary filling material (Nucavfil, Belvedere, Kent, UK), and the specimens were stored at 100% humidity and 37 °C for 2 weeks to completely set.

After 2 weeks, each tooth was embedded in self-cure acrylic resin. The root canals were cross-sectioned perpendicular to their long axes using a precision saw (Micracut 201, Metkon, Bursa, Turkey). Two slices were obtained from each tooth at depths of 1, and 5 mm and approximately 1 mm thickness. The specimens were then mounted onto glass slides.

Specimens were examined with a confocal laser scanning microscope (Zeiss LSM 510, Carl Zeiss, Göttingen, Germany) at X4 magnification with a wavelength of 488-543 nm. Digital images were imported into the Zeiss LSM Image Browser v.4.2.0 program (Carl Zeiss MicroImaging GmbH 1997-2006) to measure the dentinal tubule penetration depth and percentage of penetration. The maximum depth and percentage of sealer penetration were measured as previously described.[13]

Statistical analysis

Data were analyzed using IBM SPSS 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) statistical package program for Windows. The normality assumption was evaluated by the Shapiro-Wilk test before analysis. Numerical data were summarized with mean and standard deviation. Statistical significance of the differences between groups in terms of penetration percentage and penetration depth was evaluated by two-way analysis of variance (ANOVA). In pairwise comparisons, the Type 1 error was controlled by Bonferroni correction ($p<0.05$).

RESULTS

Representative CLSM images of each group are shown in Figure 1.

Penetration Depth Analysis

In the penetration depth analysis of 1 mm, AH Plus showed a statistically significantly higher penetration depth in the etidronic acid group than in the saline group (p=0.017). Although NeoMTA Plus was not statistically significant, it showed a higher penetration depth in groups treated with etidronic acid (Figure 2A).

In the penetration depth analysis of 5 mm, AH Plus showed statistically significantly higher penetration depth in the groups treated with etidronic acid and EDTA compared to the saline group (p=0.005). In the penetration depth analysis of 5 mm, AH Plus and NeoMTA Plus showed similar penetration depth in the etidronic acid and EDTA groups, while the average penetration depth values were low in the saline group (p<0.05) (Figure 2B).

Percentage of Penetration Analysis

In the penetration percentage analysis of 1 mm, NeoMTA Plus showed a statistically significantly higher percentage of penetration than AH Plus in the saline group (p=0.030). AH Plus showed a statistically significantly higher percentage of penetration in the EDTA group than in the saline group (p=0.032). Also, NeoMTA Plus showed a higher percentage of penetration values for each group than AH Plus, although it was not statistically significant (Figure 2C).

In the penetration percentage analysis of 5 mm, NeoMTA Plus showed a statistically significantly higher percentage of penetration than AH Plus in the saline group (p=0.001). AH Plus showed a statistically significantly higher percentage of penetration in EDTA (p=0.004) and etidronic acid (p=0.009) groups than in the saline group. Although NeoMTA Plus was not statistically significant, it showed higher penetration percentage value than AH Plus in the EDTA group. In the etidronic acid groups, it showed a similar percentage of penetration values (Figure 2D).

DISCUSSION

Some studies evaluated the effects of various chelating agents on dentinal tubule penetration by root canal sealers.[14] According to the authors' knowledge, there have been no studies investigated the effect of etidronic acid and tricalcium silicate sealers on dentinal tubule penetration. Thus, the aim of this study was to evaluate the effect of EDTA, etidronic acid, and saline solutions on dentinal tubule penetration by a tricalcium silicate-based NeoMTA Plus. It has been evaluated in comparison with AH Plus since not only the removal of the smear layer but also its physical properties can be effective in the tubule penetration of NeoMTA Plus. AH Plus, which include long-term dimensional stability, reduced

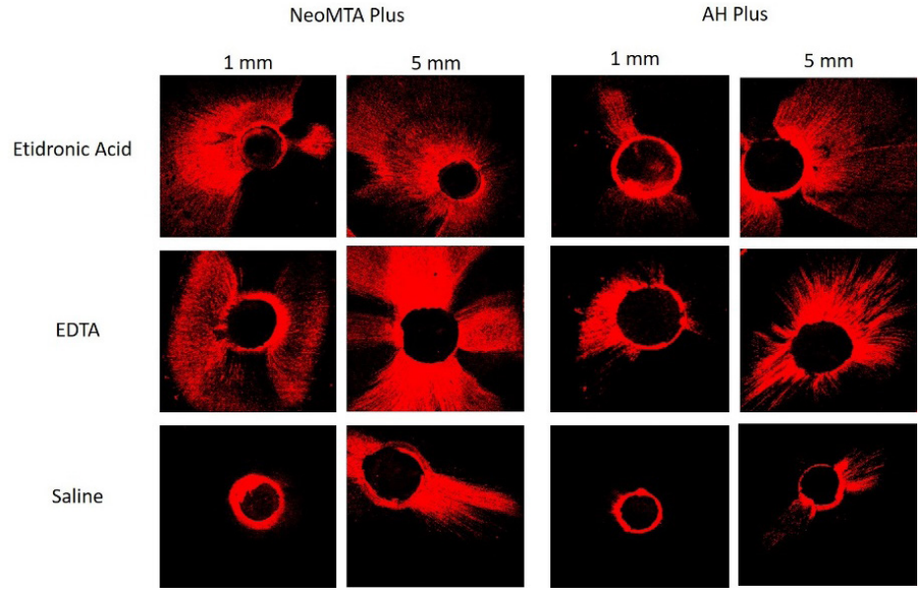

Figure 1. Representative CLSM images of each experimental group.

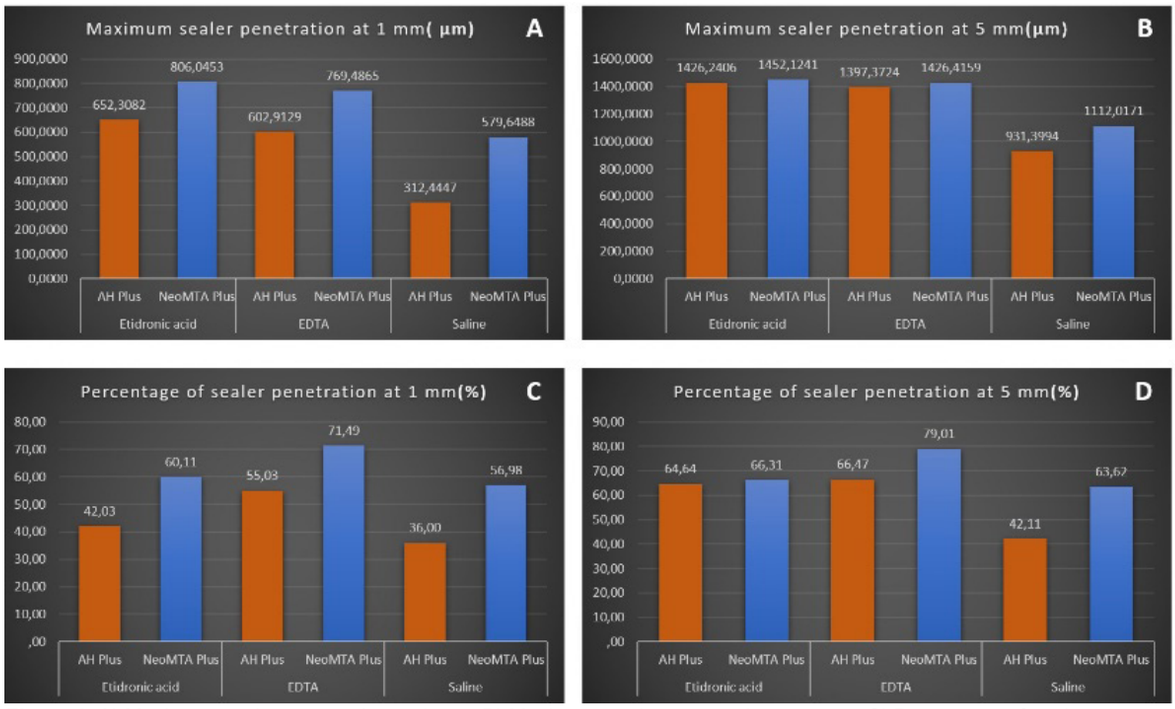

Figure 2. A) Maximum sealer penetration at 1 mm. B) Maximum sealer penetration at 5 mm. C) Percentage of sealer penetration at 1 mm. D) Percentage of sealer penetration at 5 mm.

solubility, apical adaptability, micro retention to root dentine, and low toxicity, is mostly used as a comparison material in endodontic research because of this gold standard properties.[15]

In the study, the effect of etidronic acid as another variable was evaluated comparatively with EDTA. The efficacy of the irrigating solution is dependent the chemical nature, the surface tension, the temperature, the quantity, the contact time of the irrigating solution, the type and the gauge of the needle, and the depth of penetration of the irrigation needle.[2]

It has been reported that 17% EDTA solution effectively removes the smear layer when applied to the root canals for 1 min.[16] When EDTA is applied for more than one min, it causes dentin erosion in the root canal walls, decreases the microhardness of the dentine and causes root fragility. Therefore, it is recommended to limit the application time 1 min.[3]

In the previous study was reported of the smear layer removal efficacy of 9% etidronic acid solution applied for 1 min is more effective than EDTA in the apical third.[12] Therefore, for the standardization of application times, both EDTA and etidronic acid solutions were used for 1 min in this study.

Nowadays, various obturation techniques are used to create 3 dimensional root canal obturation. However, it was emphasized that the preference of sealer should be considered when choosing the obturation technique.[17] Jeong *et al.*[18] reported that a sufficient root canal obturation can be procured when applied with a single cone technique due to the dimensional stability and fluidity of calcium silicate-based canal sealers. Thus, in this study, the single cone technique was used.

It is especially important to investigate the penetration of root canal sealers in the apical area since the apical root canal anatomy varies in terms of tubular density and size and the presence of lateral canals.[19] In several studies evaluated the dentinal tubule penetration of filling materials, the sections are generally taking from the apical and coronal of the root.[20, 21] Therefore, in the present study, the sections were taken from 1 and 5 mm.

When calcium silicate-based materials are applied to the root canals, hydroxyapatite recrystallization occurs in dentine which is called the mineral infiltration zone is a hybrid zone. In our study, some images showed the mineral infiltration zone in dentinal tubules similar to previous studies (Figure 3).[14, 18]

As stated in the previous study[14], in this study, the images were taken 14 days after obturation to allow for dynamic interactions between dentinal tissue and the calcium silicate-based sealer. However, it has not been demonstrated in the studies whether the MIZaffects the consequence of root canal treatment negatively or positively.[18]

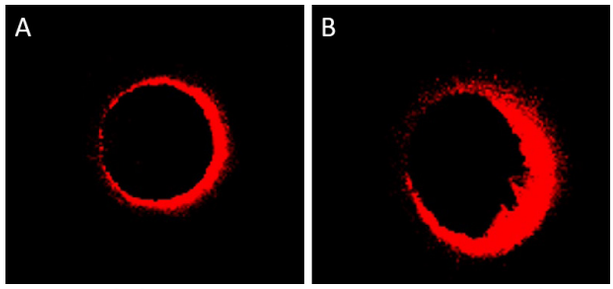

Figure 3. CLSM images representative the mineral infiltration zone of NeoMTA Plus groups. A) In the presence of EDTA. B) In the presence of etidronic acid.

When the results of this study are evaluated, the dentinal tubule penetration depth of 1 mm, both AH Plus and NeoMTA Plus showed higher penetration depth in the etidronic acid groups. However, AH Plus also showed statistical significance in the presence of etidronic acid. According to the effects of irrigation solutions on removing the smear layer, etidronic acid was more effective on the smear layer in the apical third. This result is also similar to previous studies that evaluate the smear layer removal effectiveness of etidronic acid and EDTA and this situation is based on the fact that etidronic acid has a better surface diffusion to the root canals since it has a lower surface tension than EDTA.[12, 22] Also, EDTA was a low effect on the smear layer in the apical third due to the sclerosis of the dentine in the apical third.[23]

Also, the EDTA solution, which has a neutral pH, decreases the mineral and noncollagenous protein (NCP) component of dentine. This shows that EDTA can remove not only calcium ions but also calcium bonded to NCPs. The lower degree of decalcification is observed in this part of the root dentine since the content of NCPs reduces in the apical root dentine.[24]

Another factor affecting dentinal tubule penetration of root canal sealers are their physical and chemical properties. Overall, in all irrigation solution groups, NeoMTA Plus showed a higher penetration depth than AH Plus, although not statistically significant. This may be related to the physical properties of NeoMTA Plus. Tricalcium silicate-based canal sealers such as NeoMTA Plus showed higher penetration depth due to their small particle size and high fluidity.[18, 25]

McMichael *et al.*[21] investigated the tubule penetration of tricalcium silicate-based canal sealers and reported that NeoMTA Plus when used with the single cone technique showed the highest penetration depth value at the 1 mm level. Türker *et al.*[26] evaluated the dentinal tubule penetration depth of the BioRoot RCS, MTA Plus, and AH 26 when used with the single cone technique and reported that the penetration depth of MTA Plus was significantly higher compared to BioRoot RCS and AH 26.

After irrigation of the root canals even if drying with paper points, some moisture may remain in the apical third where the narrowest part of the canal.[27] In previous study investigated the impact of residual moisture remaining in the root canal on the penetration of the epoxy resin-based canal sealer, it was reported that this moisture significantly decreased the epoxy resin sealer tubule penetration depth, especially in the apical third.[27] In the present study, the better tubule penetration of NeoMTA Plus at 1 mm than AH Plus is due to the hydrophilic structure of NeoMTA Plus.

In the analysis of penetration depth at the 5 mm level, AH Plus and NeoMTA Plus showed similar penetration depth in the etidronic acid and EDTA groups. While the etidronic acid is more effective in the removal of the smear layer in the apical third, EDTA and etidronic acid have a similar effect removal of the smear layer in the coronal third. This is thought to be due to the large tubules in the coronal third, the irrigation solutions to reach this area more easily and the chelating agents to be more effective in this area.[12, 22, 28, 29] While AH Plus showed higher penetration depth in other groups compared to the saline group, NeoMTA Plus did not differ even in saline groups, it may be due to its small particle size and fluid consistency.

In the present study, both NeoMTA Plus and AH Plus showed a higher penetration depth of the canal at the 5 mm level compared with the 1 mm level. Studies have reported increased dentinal tubule pene-

tration values in the coronal third.[29] This is because the number and diameters of dentinal tubules in the coronal third are greater than in the apical third.[30]

In the present study, both NeoMTA Plus and AH Plus showed a higher tubule penetration percentage in the presence of EDTA compared to etidronic acid at the 1 mm level. A previous study reported when 17% EDTA solution was used, despite the removal of the superficial smear layer in the apical, the dentine tubules contained moderate amounts of debris. But the surfaces of root canals and the dentine tubules in the coronal and middle thirds were free of debris.[28]

While it may be adequate to remove the superficial smear layer for percent of penetration analysis, it is important how far the irrigation solution can enter the dentine tubules and remove the smear layer within the tubules for the penetration depth. Therefore, while etidronic acid is more effective in penetration depth analysis, EDTA is more effective in percent penetration analysis at 1 mm level.

In the percentage of penetration at 1 mm, NeoMTA Plus showed higher penetration of percentage values than AH Plus for each group. NeoMTA Plus showed a higher percentage of penetration due to may have spread more around the canal circumference with its fluid consistency.

In the percentage of penetration analysis at 5 mm, although it was not statistically significant NeoMTA Plus showed higher values than AH Plus in the EDTA group. In the etidronic acid groups, it showed a similar percentage of penetration values. Hachem *et al.*[20] compared the penetration depth of tricalcium silicate based sealers and AH Plus sealer and reported that AH Plus sealer showed a lower penetration depth at 5 mm from the apex. It is being considered tricalcium silicate based sealers penetrated the deepest at this level since they have small particle sizes. In the present study, NeoMTA Plus can penetrate dentine tubules better because of its smaller particle size and fluid consistency.

With the limitations of this study, dentinal tubule penetration of NeoMTA Plus and AH Plus in the presence of EDTA and etidronic acid was evaluated using the CLSM imaging method. Accordingly, NeoMTA Plus showed better penetration into the dentine tubules due to its fluidity and small particle size. In addition, although etidronic acid contains weak acid, it is effective especially in the apical region and used compatible with NaOCl. Therefore, the use of NeoMTA Plus with etidronic acid also positively affected dentinal tubule penetration. Thus, NeoMTA Plus can be considered as an alternative to AH Plus. Further studies are needed in this direction.

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