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Original research

Micro-computed tomography assessment of triple antibiotic paste removal using different irrigation methods

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Purpose
The study aimed to compare four irrigation methods for triple antibiotic paste (TAP) removal using micro-computed tomography (micro-CT) analysis.

Materials and Methods
Forty bovine central incisor teeth were selected, and the root canals were prepared up to #6 Peeso reamer drills. Equal portions of metronidazole, ciprofloxacin, and minocycline were used for the TAP preparation. The TAP was prepared by mixing the powder with distilled water (with a powder to liquid ratio of 1 mg/1 mL). The TAP was introduced to the canals with a lentulo spiral; then, the access cavities were temporarily sealed. After 21 days of storage, the teeth were randomly divided into four equal groups according to irrigation techniques: open-ended, side-vented, double side-vented needle irrigations and EndoActivator irrigation device. The TAP was removed using 17% EDTA (20 mL) and distilled water (5 mL) for all of the groups. The volume of the intracanal medicament before and after the irrigation procedure was recorded by scanning the samples with micro-CT, and the TAP percentage was calculated. The percentages obtained from each group were compared using ANOVA. The significance level was set at $p<0.05$.

Results
The results showed that there was no statistically significant difference among the TAP percentage volumes removed by the different irrigation techniques.

Conclusion
The irrigation techniques used in this study showed similar TAP removal efficiency, however, they could not completely remove the TAP from the root canal systems.

Keywords: *Endoactivator, irrigation, regenerative endodontic treatment, side-vented irrigation needle, triple antibiotic paste*

Introduction

The immature teeth are at risk for pulp necrosis due to trauma, dental anomalies or caries; which leads the cessation of the root formation (1, 2). Regenerative endodontic treatment is an essentially recommended treatment approach for the reconstruction of a functional pulp-dentin complex in necrotic immature permanent teeth (1). This treatment approach consists of several stages including the disinfection of the root canal system, bleeding and the formation of an intracanal blood clot, and coronal sealing (3, 4). This treatment is basically conducted by disinfecting the root canal system, promoting the stem cells of the apical papilla to generate in further root development, and eliminating the signs and symptoms of infection (5). These outcomes are affected by the adequate elimination of microorganisms from the root canal space (6). The disinfecting procedure is accomplished after chemical debridement with minimal or no mechanical preparation, and the root canals are disinfected by using a medicament (7).

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Triple antibiotic paste (TAP), which is a 1:1:1 mixture of metronidazole, minocycline, and ciprofloxacin, is a commonly used medicament for regenerative endodontic protocols because of its antimicrobial efficacy (8). However, it has some drawbacks because it is difficult to totally remove the medicament from the root canals and it promotes tooth discoloration (1, 9, 10). Residual TAP may have adverse effects on the adhesion and penetration of the barrier materials to the root canal dentin (11). Moreover, the presence of residual TAP can also negatively impact the survival of the stem cells of the dental apical papilla (7, 11). In order to eliminate these negative properties, TAP should be totally removed from the root canals.

Conventional irrigation with syringes is an irrigation method that is widely accepted by both general practitioners and endodontists, although several irrigants and sonic/ultrasonic activation techniques have been introduced (12, 13). Manufacturers have designed different types of needles to make syringe irrigation more efficient, such as side-vented (SV) and double side-vented needles (DSV) (14-16). A sonic-driven irrigation solution activation system, called the EndoActivator (EA) (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA), was manufactured to produce vigorous fluid agitation in the root canals by using flexible, plastic, and non-cutting tips of various sizes (17). It has been shown that EA has a superior irrigation efficacy when compared to conventional needle irrigation (18).

In order to eliminate the negative properties of residual TAP, as stated above, it should be totally removed from the root canals. Although irrigation techniques and irrigant types are essential for the removal of the medicament, minimal or no instrumentation has been advised for regenerative procedures (3). Therefore, this study was designed to determine the optimal irrigation technique for the removal of TAP from the root canal system by evaluating the remaining TAP using a micro-computed tomography (micro-CT) system. The null hypothesis was that the removal of the TAP was not related to the irrigation technique, including open-ended (OE) needles, SV needles, DSV needles, and the EA.

Materials and Methods

Sample selection and root canal preparation

Forty bovine central incisor teeth were selected and sectioned horizontally to standardize the root lengths at 17 mm. The working length was established at 16 mm. The root canals were instrumented with using hand instrumentation technique up to the #80 K- file, and they were prepared with #1-6 Peeso reamer drills. At each instrument change, 2 mL of 1.5% sodium hypochlorite (NaOCl) was used for irrigation. After finishing the instrumentation protocol, 20 mL of 1.5% NaOCl (5 min), 5 mL of distilled water, and 20 mL of 17% ethylenediaminetetraacetic (EDTA) (5 min) were applied to the root canals. Then, the root canals were dried using paper points (Dentsply Maillefer, Ballaigues, Switzerland). Equal portions of metronidazole (Eczacibasi, Istanbul, Turkey), ciprofloxacin (Biofarma, Istanbul, Turkey), and minocycline (Ratiopharm, Ulm, Germany) were used for the TAP preparation. The medicament was prepared by mixing the powder with distilled water (with a powder to liquid ratio of 1 mg/1 mL) and it was introduced into the root canals with a Lentulo spiral at 900 rpm until the medicament extrusion was visible at the apical foramen. The apical portions of the canals were sealed with a flowable composite resin, and the access cavities were temporarily sealed (Cavit G; 3M ESPE, Seefeld, Germany). The teeth were stored at 37 °C and 100% humidity.

Medicament removal

The teeth were randomly assigned to one of four groups. After 21 days of storage, the intracanal medicaments were removed via irrigation with 17% EDTA (20 mL, 4 min) and distilled water (5 mL, 1 min) as follows: OE needle irrigation: A 27 G beveled OE dental irrigation needle (Ayset, Adana, Turkey) was used for the irrigation procedure. SV needle irrigation: A 30 G SV irrigation needle (Max-i-Probe; Dentsply Maillefer, OK, USA) was used for irrigation procedure. DSV needle irrigation: A 30 G DSV needle (i-Tips; i dental, Siauliai, Lithuania) was used for the irrigation procedure. EA irrigation: A 2.5 mL of the irrigation solution was flushed into the canal by using a 27 G needle and it was activated with the red tip of the EA (25/.04) at 10,000 cycles/min. After every 2.5 mL of irrigation, the irrigant was activated for 30 seconds.

The tips/needles were moved with an up and down motion in all of the groups. The maximum depth of the tips/needles was positioned at 2 mm short of the apical foramen. During the irrigation procedures, each canal was flushed with a 2.5 mL of irrigant for a total of 30 seconds. Therefore, 20 mL of EDTA solution was applied in 4 min, and 5 mL of distilled water was applied in 1 min. The same amount of irrigant and the same irrigation time were applied to every root.

Micro-computed tomography evaluation

For the volumetric analysis of the filling materials, the teeth were scanned using micro-CT (SkyScan 1174; Bruker micro-CT, Kontich, Belgium) before and after the irrigation protocols were performed with the following scanning conditions: 50 kVp, 800 µA, a pixel size of 33 um, a beam hardening correction of 30%, a smoothing of 2 and a ring artifact correction of 6. The scanning was performed with a 180° rotation around the vertical axis, a camera exposure time of 2.700 ms, a rotation step of 0.4°, and a frame averaging of 3. Flat field corrections and geometric corrections for random movement were performed in all of the scans. The scanning procedure took approximately 1 h per sample.

The three-dimensional reconstruction data was obtained by using NRecon reconstruction software (version 1.6.9.4; Bruker micro-CT). Serial section images obtained with NRecon software were opened in CTAn program (version 1.17.7.2; Bruker micro-CT) for the calculation of TAP volume. The region of interest (ROI) area was determined to measure the TAP volume. Care was taken to position the ROI between the root canal wall and the tooth surface. This situation has been checked for all sections. Dental tissue and TAP were clarified by adjusting the upper and lower values from the histogram section in the CTAn software. The area included for the measurement of the TAP volume (TAP volume = volume of interest-VOI) was shown with the red colour. The green and black coloured areas in the figure are not included in the measurement. These settings

were applied for all sections by selecting the from dataset option. The CTAn software provided the TAP area. No methodological modifications were applied. A 3D model of TAP was created with the Create 3D model option. In order to create a 3D model of the tooth with TAP, the ROI area was determined again with the same sections. The ROI was positioned outside of the tooth surface. All measurements were performed with these settings. Only the tooth tissue was included in the VOI by adjusting the upper and lower values from the histogram section in the CTAn software. This could be made by the difference in radiopacity between the tooth and TAP. The green and black areas in the figure were not included in the measurement, the red area was included in the measurement (VOI = tooth volume). These settings were applied for all sections by selecting the from dataset option. A 3D model of tooth was created with the Create 3D model option. All of the created 3D files were opened using the CTVol software (version 3.3.0; Bruker micro-CT). The three-dimensional visualization and qualitative evaluation of the TAP were performed using CTVol software. TAP was coloured to make it more distinct and the tooth was made more transparent to provide TAP to be seen in the tooth (Figure 1 A-B). TAP volume image was fitted into the tooth model. All these procedures were performed using opacity and colors settings in the objects menu of CTVol. The examination of the images was performed by a blinded observer. The volume of the TAP before and after the irrigation procedure was recorded, and the TAP percentage was calculated (Figure 1 A-B). The horizontal sections of the teeth were obtained using DataViewer software. Black and white images were available as raw data obtained from the scanning (Figure 1 C-E). To ease the TAP detection, the black and white raw images were colourized in the DataViewer program (Figure 1- D-F). DataViewer created this color difference owing to the radiopacity difference.

Statistical analysis

The Shapiro-Wilk test was used to evaluate the assumption of normality. The pre-operative TAP volume for the groups showed a non-normal distribution ($p < 0.05$). The percentage volumes of the remnant TAP for each irrigation technique were normally distributed. The pre-operative TAP volumes of the groups were compared with Kruskal-Wallis test. The percentages obtained from each group were compared using ANOVA. The significance level was set at $P < 0.05$ (IBM SPSS Statistics for Windows, Version 22.0; IBM Corp., Armonk, NY, USA).

Results

There was no statistically significant difference among the techniques in the TAP residue percentages.The statistical results and the volumes of the intracanal medicament before and after the irrigation procedure are shown in Table 1. The 3D and 2D images taken before and after removal of TAP for

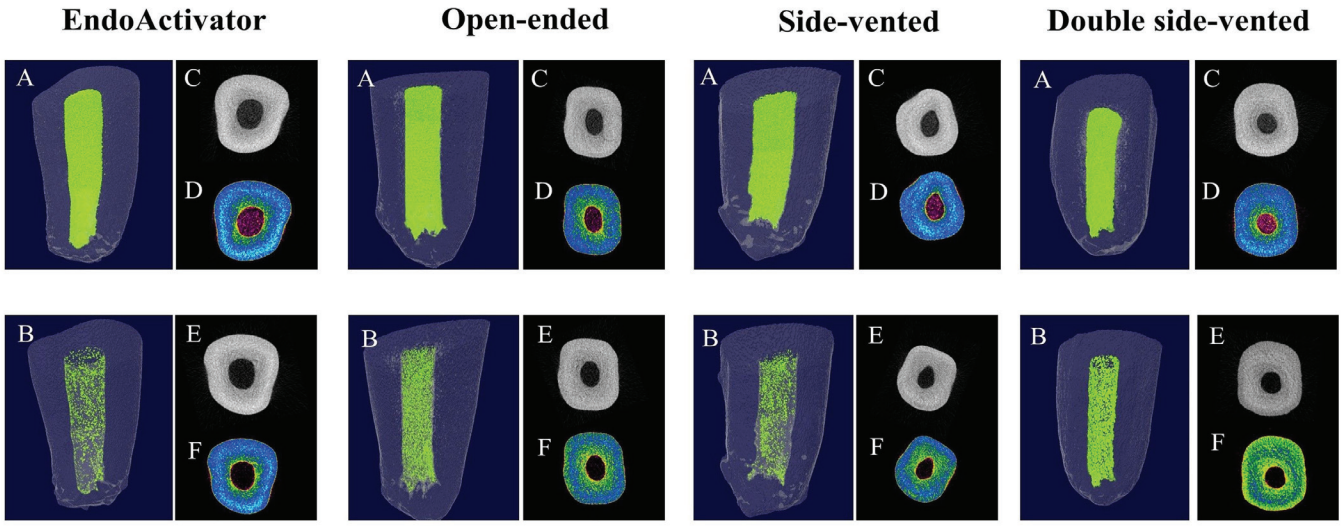

Figure 1. *Images obtained with different observational methods. 3D images observed (A) before, and (B) after TAP removal. (C) Black and white, (D) coloured cross-sectional 2D images taken before TAP removal. (E) Black and white, (F) coloured cross-sectional 2D images taken after TAP removal.*

Table 1. *TAP volumes before and after removing procedures, and comparison of residual TAP percentages for groups (ANOVA)*

Groups	TAP volume before removal (mm³)		TAP volume after removal (mm³)		TAP percentage (%)	F	*P*
	Mean-Median	Min-Max	Mean-Median	Min-Max	Mean±sd		
OE	58.46-53.68	27.34-106.21	9.11-9.01	6.76-12.69	7.05±1.16	0.321	0.810
SV	65.82-62.93	19.02-164.85	7.76-7.22	6.05-10.54	5.82±0.72		
DSV	44.85-30.06	19.99-90.48	7.12-6.54	4.74-11.28	6.97±1.37		
EA	58.35-28.71	24.18-140.84	7.43-7.62	4.35-11.40	7.41±1.46		

sd: standard deviation; Min: minimum; Max: Maximum; TAP: Triple antibiotic paste; OE: Open-ended needle irrigation; SV: Side-vented needle irrigation; DSV: Double side vented needle irrigation; EA: EndoActivator irrigation.

each technique are represented in Figure 1. No statistically significant difference was found among the groups for pre-operative TAP volumes (chi-square = 2.129, P = 0.546).

Discussion

This study was designed to compare the efficacy of OE, SV, DSV needles, and the EA technique for removing TAP from root canals using a micro-CT imaging system. The tested null hypothesis was accepted because there was no statistically significant difference among the tested irrigation methods.

Dental trauma to immature teeth could lead the cessation of the root formation and thinner root canal walls (19). The root canal spaces of immature teeth are larger than the canal spaces of mature teeth requiring regenerative endodontic treatment. In this study, the root canals were prepared with Peeso reamer drills up #6 in order to provide larger canals to simulate the clinical conditions of immature teeth. In some previous endodontic studies, bovine teeth had been used, since the bovine dentine has a similar structure, chemical composition and number of tubules to human root dentin (20-23). It was found that, the adhesion ability of different sealers to the human and bovine dentin were similar (22). Instead of using human teeth, bovine incisors were used in this study because of the mentioned reasons.

The intracanal application of medicaments is a powerful way to combat pulp necrosis pathogens. More specifically, TAP has been recommended for regenerative procedures because its success with eliminating the endodontic infections has been proven (2, 24). Although TAP provides a significant disinfection, it has some disadvantages, such as notable tooth discoloration and significant stem cell death, especially at dense concentrations (2, 7, 25, 26). Because it has been proven that the use of TAP at 1 mg/mL has no adverse effects on cell survival, the TAP was prepared at that concentration in this study in order to mimic the clinical conditions (6). EDTA has been shown to remove TAP effectively from root canals, and also release growth factors from the dentin, and support the adhesion, migration, and differentiation of the stem cells of the dental pulp (27, 28). For the TAP removal, the irrigation procedure was performed with 17% EDTA and distilled water as suggested (29). The maximum depth of the tips/needles was maintained at 2 mm above the apical foramen during the irrigation procedure based on the recommendations of European Society of Endodontology (ESE) for revitalization procedures (29).

Residual TAP is a challenge for regenerative procedures as it acts as a barrier between filling material and root canal walls. Therefore, the medicament should be efficiently removed. However, current study showed that TAP can not be totally removed, regardless of the irrigation technique. TAP was proved to have higher diffusion and retention capacities toward the dentin tubules, induced by the chelation of calcium ions by minocycline which probably caused the lack of adequate medicament removal (1).

One study compared the needle types via a computational fluid dynamics model, which showed that the flow conditions of OE and close-ended needles were different (30). It was stated that the shear stress observed with SV or DSV needles increases on the root canal walls, which means they achieve a higher debridement efficacy. However, different from this study, that study was created on a simulated 6% tapered root canal model. In this study, the canals were not tapered, and the widths of the root canal spaces were standardized by preparing them with #6 Peeso-reamer drills to mimic the enlarged root canals of immature teeth. Therefore, the similarity of the results obtained from all of the groups could be related to the untapered shape of the root canals, which may have allowed irrigation solution to easily flow back and remove the apical medicament to the coronal portion of the root canal.

Previous studies that compared EA and classic needle irrigation techniques for removal of the antibiotic paste and root canal sealer found EA more effective (31-33). EA has a non-cutting tip, and the tip generates short vertical strokes by vibration and up-and down movements, which ease the elimination of debris from the root canals (18). Activating the irrigation solutions are claimed to ease the irrigation flow and therefore cleaning the paste from the root canal walls. In these studies, the mature teeth with tapered root canals which had been preparing up to #40 apical size were used. It has been claimed that an increase in the taper of the root canals facilitates the irrigant flow, and debridement from the apical to the coronal part of the root canals (34). In this study, the teeth were prepared as non-tapered form, similar to a cylinder. The similarity among the techniques may depend on the shape of the root canals.

In some previous studies that evaluated several irrigation methods or solutions for antibiotic paste removal, a stereomicroscope was used for the determination of the remnant medicament (11, 27, 31, 32, 35-39). In another study, radio-labeled TAP was introduced into the root canals, and the residual material was evaluated radiographically (1). In stereomicroscopic evaluation, the roots are longitudinally sectioned and residual material is scored, or the areas of remnant material and root canal surface are measured on the images. The sectioning procedure may cause the remnant material removal and thus causing misleading results. The stereomicroscopic and radiographic evaluations are both made on 2D images. The micro-CT imaging lets volumetric calculations on 3D imaging, and previously used to evaluate the effects of irrigation techniques on removal of filling material and calcium hydroxide from the root canals (40, 41). Previous studies using micro-CT imaging to evaluate volumetric solubility of TAP used artificial fabricated resin acrylic roots (42, 43). In this study, bovine teeth were used to simulate clinical conditions such as the bonding between TAP and dentin, and the effect of irrigation techniques to remove paste from dentinal walls. However, the radiopacity level of TAP may not be enough for micro-CT imaging, and this could be a limitation for the current study.

It has been claimed that irrigation tips lead to apical extrusion, which may cause some complications, such as flare-ups, periapical inflammation, and the delayed healing of apical lesions (14). Causing less debris extrusion may be another important factor for immature teeth in order to prevent the TAP from going beyond the apical foramen. Several studies have compared the techniques used in this study with regard to apical extrusion at mature teeth (14, 44-46). Because this study proved that the irrigation techniques had similar effects with regard to TAP removal, another important factor, apical extrusion, could affect the selection of the irrigation method. Further studies evaluating debris extrusion using

these methods with immature teeth may help to specify the optimum irrigation technique for immature teeth.

Conclusion

Although the SV needle irrigation method provided a lower remnant material percentage; the OE, SV, DSV and EA irrigation methods left statistically similar amounts of TAP in the root canals. None of the methods investigated were able to totally remove the TAP from the root canal system.

Türkçe Özet: *Farklı irrigasyon yöntemlerinin üçlü antibiyotik patı uzaklaştırma etkinliğinin mikro-bilgisayarlı tomografi ile değerlendirmesi. Amaç: Bu çalışmanın amacı, dört farklı irrigasyon tekniğini, üçlü antibiyotik patını (ÜAP) uzaklaştırma etkinliği açısından mikro bilgisayarlı tomografi (mikro-BT) yöntemi ile karşılaştırmaktır. Gereç ve Yöntemler: Kırk adet sığır keser dişi seçildi ve kök kanalları # 6 Peeso reamer kalınlığına kadar genişletildi. ÜAP tozu için eşit miktarda metronidazol, siprofloksasin ve minosiklin kullanıldı. ÜAP tozunun distile suyla (1 mg / 1 mL toz-likit oranında) karıştırılmasıyla pat elde edildi. ÜAP, lentülo spiral ile kanallara iletildi, ardından giriş kaviteleri geçici olarak kapatıldı. Yirmi bir gün sonra, dişler irrigasyon tekniğine göre rastgele dört eşit gruba ayrıldı: açık uçlu, tek yandan delikli, ve çift yandan delikli irrigasyon iğneleri ve EndoActivator irrigasyon cihazı. ÜAP, tüm gruplarda % 17 EDTA (20 mL) ve distile su (5 mL) irrigasyonu ile uzaklaştırıldı. Kanal içi medikaman hacmi, irrigasyon işleminden önce ve sonra mikro-BT cihazı ile taranarak kaydedildi ve artık ÜAP yüzdesi hesaplandı. Bulgular: Farklı irrigasyon teknikleriyle uzaklaştırılan ÜAP yüzdesi hacimleri arasında istatistiksel olarak anlamlı bir fark olmadığı görüldü. Sonuç: Bu çalışmada kullanılan irrigasyon teknikleri ÜAP uzaklaştırma etkinliği açısından benzerlik gösterdi, ancak hiçbir sistem kök kanal sisteminden patı tamamen uzaklaştıramadı. Anahtar kelimeler: endoactivator; irrigasyon, rejeneratif endodontik tedavi; yandan delikli irrigasyon iğnesi, üçlü antibiyotik patı.*

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