Influence of access cavity preparation on calcium hydroxide removal using different cleaning protocols: A confocal laser scanning microscopy study

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Research Article

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Abstract

Objectives

The purpose of this study was to evaluate the influence of endodontic access cavities design on the removal of calcium hydroxide medication of the apical third of mandibular incisor root canal walls and dentinal tubules with different cleaning protocols: EDDY sonic activation, Er,Cr:YSGG laser-activated irrigation, or conventional irrigation with IrriFlex.

Materials and Methods

Seventy eight extracted human mandibular incisors were assigned to six experimental groups [n = 13] according to the endodontic access cavity and cleaning protocol for calcium hydroxide removal: traditional access cavity (TradAC)/EDDY; ultra-conservative access cavity performed in the incisal edge (UltraAC.Inc)/EDDY; TradAC/Er,Cr:YSGG; UltraAC.Inc/Er,Cr:YSGG; TradAC/IrriFlex; or UltraAC.Inc/IrriFlex. Confocal laser scanning microscopy images were used to measure the non-penetration percentage, maximum residual calcium hydroxide penetration depth, and penetration area at 2 and 4 mm from the apex. Data were statistically analyzed using Shapiro–Wilk and WRS2 package for two-way comparison of non-normally distributed parameters (depth of penetration, area of penetration, and percentage of non-penetration) according to cavity and cleaning protocol with the significance level set at 5%.

Results

The effect of cavity and cleaning protocol interactions on penetration depth, penetration area and non-penetration percentage was not found statistically significant at 2 and 4 mm level ($p > 0.05$).

Conclusion

The present study demonstrated that TradAC or UltraAC.Inc preparations with different cleaning protocols in extracted mandibular incisors did not influence the remaining calcium hydroxide at 2 and 4 mm from the apex.

Clinical Relevance

This study highlighted that TradAC or UltraAC.Inc, access cavities design and the different cleaning protocols have not influenced the remaining calcium hydroxide apical third of mandibular incisor.

Introduction

Access cavity preparation is the foremost technical step of root canal treatement requiring extensive knowledge of the internal and external anatomy of the teeth. The traditional access cavity (TradAC) preparation focuses in three key points: [i] complete unroofing of the pulp chamber with the exposure of the pulp horns, [ii] creation of a smooth unimpeded pathway to the root canal orificies, and [iii] preservation of sound structure of the tooth [1–4]. In an attempt to minimize the removal of tooth structure, the conservative access cavity (ConsAC) and ultraconservative access cavity (UltraAC) preparation emerged targeting the maintance of the pulp chamber roof and the pericervical dentine as much as possible in order to ultimately improve the tooth´s survival [5–8]. While some studies demonstrated an improved fracture resistance in teeth with ConsAC and UltraAC [9, 10], the majority of studies failed to demonstrate such an effect [5, 11–13]. In addition, it has been reported that such minimally invasive access cavities might compromise proper instrumentation, cleaning, and disinfection of root canals [6].

Calcium hydroxide has been extensively used in Endodontics to improve root canal disinfection [14]. One important concern regarding its use is related to difficulties in removing from the root canal system [15, 16]. As calcium hydroxide residues can affect the penetration of filling material into the dentinal tubules and increase apical leakage [17] a complete removal is desired. Therefore, the combination of chemical effect and mechanical activation of the irrigant has been proposed to ensure favorable results [18].

The EDDY (VDW, Munich, Germany) tip is made from flexible polyamide with a size of 25.04 to prevent it from cutting dentin and changing root canal morphology during sonic activation at high frequency [6000 Hz]. According to the manufacturer, it allows an efficient cleaning of complex root canal systems without the limitations of ultrasound-activated devices. Studies on the effectiveness of EDDY in removing calcium hydroxide from root canals indicated that it is successful in the apical region [19–21]. Laser-activated irrigation [LAI] using Erbium, Chromium:Yttrium-Scandium-Gallium-Garnet (Er,Cr:YSGG) has been used as an alternative method to release the irrigant more deeply, thereby increasing the cleaning ability inside the root canal system [22]. LAI increases debris, smear layer and calcium hydroxide removal from the apical third of the root canal system [23–25]. IrriFlex needle (Produits Dentaires SA, Switzerland) is a flexible root canal irrigation needle. It has two side vents at the tip, back-to-back with a size of 30 G (0.3 mm) and 0.04 taper (www.pd-irriflex.com). This unique characteristic gives balanced irrigant expulsion, via two accurate jets, oriented directly to the dentinal walls. According to manufacturer, the flexibility of the polypropylene body allows the needle to access the apical region without resistance or damage to the dentinal walls. The tapered shape of the needle adapts to the shape of the canal. Flow thickness of the irrigant is therefore constant as the fluid moves to the coronal area, which maximises shear forces and the elimination of debris, smear layer and biofilm [26].

This study aimed to assess the influence of access cavity preparation on calcium hydroxide removal from the apical third of mandibular incisor root canals using different cleaning protocols: sonic activation, Er,Cr:YSGG LAI and conventional irrigation with IrriFlex. The null hypotheses of the present study were as follows:

1. The access cavity design would have no effect in the removal of calcium hydroxide;

2. There would be no differences in calcium hydroxide removal among the different cleaning protocols.

Materials And Methods

The sample size of 13 samples per group was determined after a pilot study (power = 0.95, effect size = 0.66, alpha-type error = 0.05).

Seventy eight extracted human mandibular incisors with intact apices, a minimum tooth length of 18 mm and no previous treatments were selected from a random collection of extracted teeth. Radiographs of all teeth were obtained from bucco-lingual and mesio-distal projections and used to select teeth with a single oval-shaped canal [7]. The teeth were numbered and randomly allocated (http://www.random.org) into 2 groups (n = 39) according to the following acces cavity designs:

Ultraconservative access cavity performed in the incisal edge (UltraAC.Inc)

UltraAC.Inc were drilled with diamond burs (802L 314 – 012; Dr. Hopf GmbH & Co. KG, Langenhagen, Germany) under water cooling at high speed. Incisors were accessed 1 mm palatal to the incisal edge, and cavities extended apically along the long axis. Canals were located while minimizing mesial-distal, buccal-lingual, and circumferential pericervical dentin removal [27] (Fig. 1a **and c**).

Traditional access cavity (TradAC)

TradAC were prepared following conventional guidelines [3] for outline size and using round 1014 HL and Endo-Z burs (Dentsply Maillefer, Ballaigues, Switzerland) at high speed under water cooling (Fig. 1b **and d**).

After access cavity preparation, the root canals were instrumented using TruNatomy Glider (17, 0.02v taper), Small (20,0.04v taper), Prime (26,0.04v taper) instruments at 500 rpm and 1.5 Ncm torque values. During the canal preparation, the root canal was irrigated with 2.5 mL 2.5% NaOCl solution after each instrument. After completion of the mechanical preparation, a final irrigation was applied using 5 mL 17% EDTA followed by 5 mL 2.5% NaOCl, and 5 mL distilled water. Them, the canal was dried with paper points.

The root canals were filled with a paste made of calcium hydroxide powder (Kalsin; Spot Dis Deposu A.S.; Izmir, Turkey) mixed with saline solution and 0.1% rhodamine B dye (Sigma-Aldrich, St Louis, MO). Two radiographs (mesiodistal and buccopalatal direction) were taken to confirm complete filling of the canals with the calcium hydroxide paste. The access cavities were sealed with Cavit (3M ESPE, Seefeld, Germany), and the specimens were stored for 1 week at 37 ^{o}C in 100% relative humidity. After this period, two coats of colored nail polish were applied to the specimens, including the apical foramen, to prevent leakage of the irrigant. The specimens were fixed in Eppendorf vials with silicone impression material (Optosil; Heraeus Kulzer; Hanau, Germany). These procedures were performed to create a closed system, simulating the vapor lock effect [28]. The roots were divided into 3 subgroups according to the irrigation protocol, as follows:

Sonic Activation with EDDY

Before each cycle of activation, 2.5 mL 17% EDTA (Cerkamed, Stalowa, Poland) was applied to the root canal with a syringe. The irrigant was activated with a frequency of 6000 Hz and an amplitude of 160 mm using an air scaler (TA-200-S4H, MICRON Co., Tokyo, Japan). The EDDY tip was placed 2 mm short of the WL, and in-and-out movements with an amplitude of 5 mm were performed.

Laser-activated irrigation with Er,Cr:YSGG: The root canals were filled with 2.5 mL 17% EDTA and activated with Er,Cr:YSGG laser (Waterlase MD, Biolase Technology Inc., San Clemente, CA, USA) using the RFT2 tip (275 microns in diameter and 21 mm length), placed 2 mm short of the WL. The parameters of laser used were output power of 3W energy, pulse frequency of 20 Hz (pulses per second), using 10% air and 10% water [29]. The irrigant was not aspirated from the canal.

IrriFlex irrigation

A 2.5 mL syringe with 30-G IrriFlex needle was placed 2 mm short of the WL into the canal, and in-and-out movements with an amplitude of 5 mm were performed; 17% EDTA was applied over 30 s.

In all groups, irrigation activation was repeated two times for 30 seconds, resulting in a total of 1 minute of irrigation with 5 mL EDTA. Then, final irrigation with 5 mL 2.5% NaOCl and 5 mL distilled water was performed with IrriFlex needle. After removal from the impression material, each specimen was embedded in a circular self-cure acrylic resin mold. Two 1-mm thick sections of each tooth were obtained at distances of 2 and 4 mm from the root apex using a slow-speed, water-cooled 0.3-mm microtome saw (Isomet 5000; Buehler, Lake Bluff, IL). Slices were taken for confocal laser scanning microscopy (CLSM; Zeiss LSM 800, Jena, Germany) at x5 magnification with a laser wavelength of 561 nm. Excitation wavelength was set at 543 nm, and emission wavelength was set at 565 nm. These images were used to measure the non-penetration percentage, maximum residual calcium hydroxide penetration depth and penetration area (Fig. 2). The method proposed to measure the penetration percentage by Moon et al. was adapted to measure the percentage of clean root canal walls [30]. First, in each sample, the total perimeter of the root canal was measured with the Zeiss Zen software (Carl Zeiss, Jena, Germany) measuring tool. Then, the perimeter along the root canal walls where there was no evidence of residual calcium hydroxide was measured (Fig. 2a). Next, this value was divided by the perimeter of the root canal and this result was multiplied by 100 to calculate the percentage. For the maximum depth of residual calcium hydroxide into the dentinal tubules, the point from the root canal wall to the deepest point where residual calcium hydroxide could be observed was measured at four predetermined points (mesial, distal, buccal and lingual). The mean depth of residual calcium hydroxide was measured by averaging these 4 values for each specimen (Fig. 2b). Penetration area of residual calcium hydroxide was measured using the area calculating tool of the software (Fig. 2c). These measurements were made by an investigator who was blind to the experimental groups.

Statistical Analysis

The data were analyzed with the R Project program (version 3.5.0; Vienna, Austria). Conformity to normal distribution was evaluated using the Shapiro Wilk test. WRS2 package [31] was used for two-way comparison of non-normally distributed parameters (depth of penetration, area of penetration and percentage of non-penetration) according to cavity and cleaning protocol. Significance level was taken as $p < 0.05$.

Results

The effect of cavity and cleaning protocol interactions on penetration depth, penetration area and non-penetration percentage was not found statistically significant at 2 and 4 mm level ($p > 0.05$) (Fig. 3). Figure 4 shows representative images of the apical root third of specimens.

Discussion

Minimally invasive access cavities influence on different outcomes have been extensevely evaluated in recent years, with indefinite conclusions mainly those related to the fracture resistance [8]. However, to the best of the authors knowledge, the influence that TradAC and UltraAC.Inc preparations have in the removal of calcium hydroxide medications, evaluated using CLSM, was not reported yet. The present study design aimed to assess the influence of access cavity preparation on calcium hydroxide removal from the apical third of mandibular incisor root canals using different cleaning protocols: sonic activation, Er,Cr:YSGG LAI and conventional irrigation with IrriFlex. The results of the present study demonstrated no differences among all tested groups irrespective the access cavity preparation or cleaning protocols. Therefore, the first and second null hypothesis were accepted.

Complete removal calcium hydroxide of from apical third of root canals was not achieved in any of the access cavity preparation design or any of the cleaning protocols. Several authors observed that the apical third of the root canal is the most affected by remnants of calcium hydroxide [28, 32]. Göktürk et al. [33] demonstrated that the apical third exhibited higher amounts of residual calcium hydroxide than the coronal and middle thirds of the root canals. For this reason, 2 and 4 mm sections from the apical third were examined in this study.

CLSM was used to evaluate calcium hydroxide removal, similar to the previously published only one study [28]. As the residues of calcium hydroxide have small particle size it might penetrate into dentinal tubules [34]. CLSM offers several advantages over conventional microscopic techniques for evaluating the dentinal tubule penetration of endodontic materials. For example, penetration of the calcium hydroxide can be assessed more quickly, and a detailed view of the specimen can be obtained at lower magnifications using fluorescent Rhodamine B dye [35].

In the present study, the different designs of the endodontic access cavity of mandibular incisors did not interfere in removing calcium hydroxide outcomes, regardless of the irrigation techniques used. Previous studies demonstrated no influence of minimally invasive endodontic access cavities in the shaping ability of mandibular incisors [7, 36]. In the current study, similar to the previously published studies, the selected mandibular incisors had only one root canal, with small dimensions, and absence of curvatures.

Undoubtably, such simplistic anatomy might corroborate with the results. Moreover, the straight-line access may have contributed with the current results. Similar to the present results, Eren and Özyürek [37] reported that the conservative and traditional endodontic cavities did not affect the removal of calcium hydroxide from artificial groves in the coronal and apical portions mandibular premolars. However, these authors used stereomicroscope methodology, which does not allow the evaluate calcium hydroxide medication inside dentinal tubules. Future studies should be performed aiming to evaluate calcium hydroxide removal in complex root canal anatomies.

Each one of the used irrigation techniques have different fluid dynamics characteristics, and no studies compared these techniques before. Previously published studies compared the calcium hydroxide removal efficiency of EDDY with different irrigation techniques and demonstrated better results for removal using EDDY [19–21]. When compared to the present, these different results might be explained by differences in the methodology. In these three studies, the evaluation of calcium hydroxide removal was performed in artificial grooves with a laser scanning microscope [19], optical microscopy and scanning electron microscopy [20], and stereomicroscope [21]. However, the lack of irregularities in artificial grooves prepared on root dentin probably facilitated the dissolution and removal of the calcium hydroxide. In addition, Rödig et al. [38] reported no differences when EDDY activated irrigation was compared to manual irrigation on the ability to remove accumulated hard-tissue debris in the mesial root canal systems of mandibular molars. Authors emphasized that the absence of differences might be a result of the needle insertion depth at 2 mm from WL. In the present study, all irrigation devices tips were placed at 2 mm short of the WL. It is also important to emphasize that manual irrigation was performed with IrriFlex needle, which has improved flexibility allowing access in the apical portion of the root canal.

LAI is based on the transmission of energy that produces transient cavitation in the liquid through optical breakdown [39]. Abduljalil and Kalender [29] reported that using the Er,Cr:YSGG laser at the parameters of 2780 nm, 20 Hz, and 3.0 W was significantly more effective in removing the filling remnants than at the 1.5 W output power. Therefore, these parameters were used in the present study. Eymirli et al. [25] reported that needle or laser-assisted irrigation modes could remove calcium hydroxide completely, but LAI was more efficient than 27-G needle irrigation in artificial grooves evaluated with a stereomicroscope. However, this study performed manual irrigation with a 30-G IrriFlex needle and observed no differences between LAI and IrriFlex. Different methodology and the needle tip size may have interfered with irrigation performance.

Conclusion

Within the current results access cavity design and cleaning protocol did not affect the penetration depth, penetration area, and non-penetration percentage of calcium hydroxide in CLSM images of mandibular incisors apical region.

Declarations

Funding No funding was obtained for this study.

Conflicts of Interest The authors deny any conflicts of interest related to this study.

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Ethical approval The local ethics committee approved the experimental protocol of this study (No: DÜ/2021-12).

Conceptualization; Seda Falakaloğlu, Merve Yeniçeri Özata, Betül Güneş Data curation; Seda Falakaloğlu, Merve Yeniçeri Özata, Betül Güneş Formal analysis; Seda Falakaloğlu, Merve Yeniçeri Özata, Betül Güneş Funding acquisition; Seda Falakaloğlu, BurcuGüçyetmez Topal Investigation; Seda Falakaloğlu, Emmanuel JNL Silva, Mustafa Gündoğar Methodology; Seda Falakaloğlu, Merve Yeniçeri Özata, Betül Güneş Project administration; Seda Falakaloğlu, Burcu Güçyetmez Topal Resources; Seda Falakaloğlu, Emmanuel JNL Silva Software; Seda Falakaloğlu, Merve Yeniçeri Özata, Betül Güneş Supervision; Emmanuel JNL Silva Validation; Seda Falakaloğlu, Merve Yeniçeri Özata, Betül Güneş, Emmanuel JNL Silva, Mustafa Gündoğar, Burcu Güçyetmez Topal Visualization; Seda Falakaloğlu, Merve Yeniçeri Özata, Betül Güneş, Emmanuel JNL Silva Roles/Writing-original draft; Seda Falakaloğlu, Emmanuel JNL Silva, Mustafa Gündoğar Writing-review &editing; Seda Falakaloğlu,Emmanuel JNL Silva, Mustafa Gündoğar.

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Figures

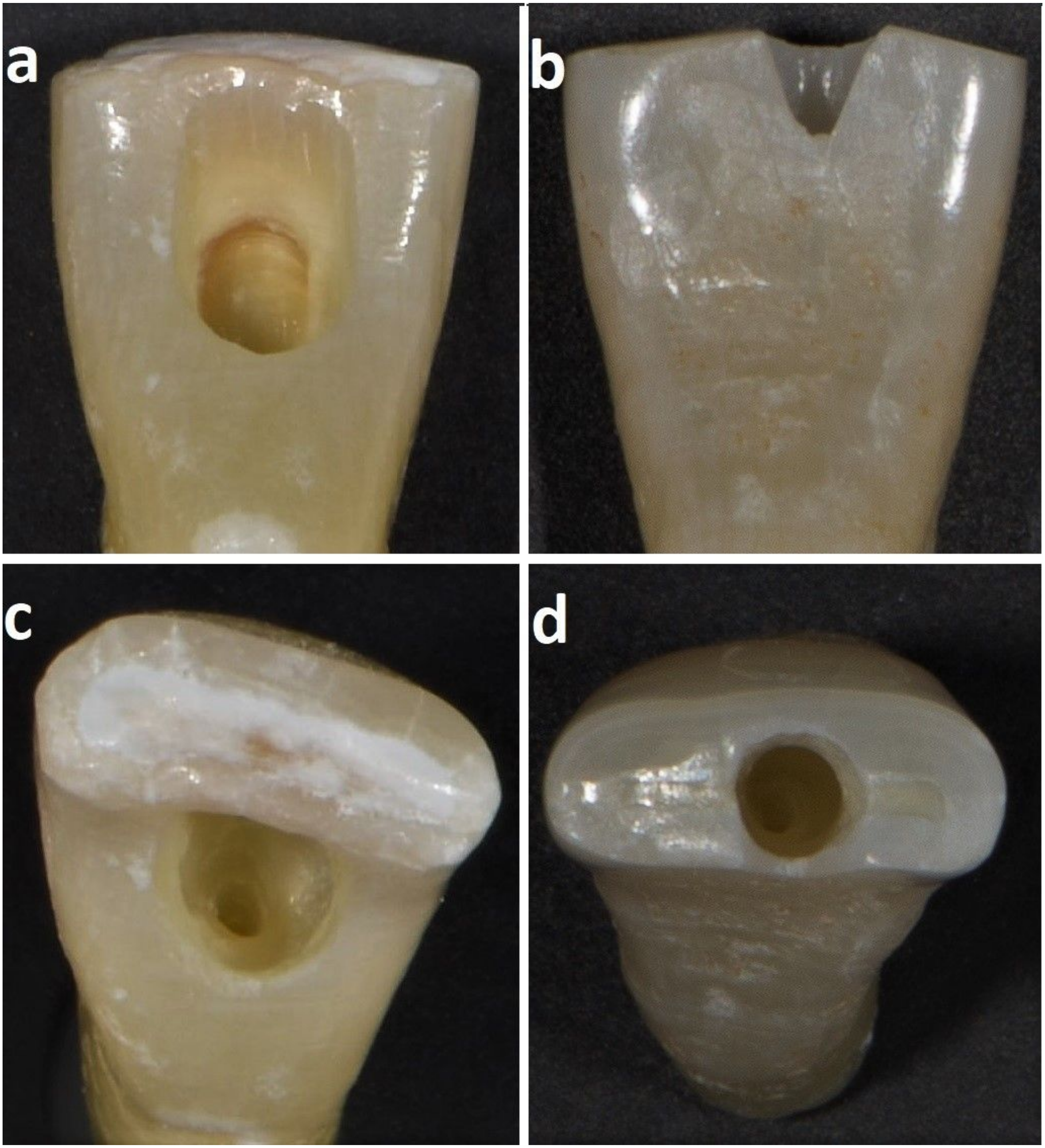

Figure 1

Photographs demonstrating the cavity designs [a and c] Traditional access cavity; and [b and d] Ultraconservative access cavity performed in the incisal edge.

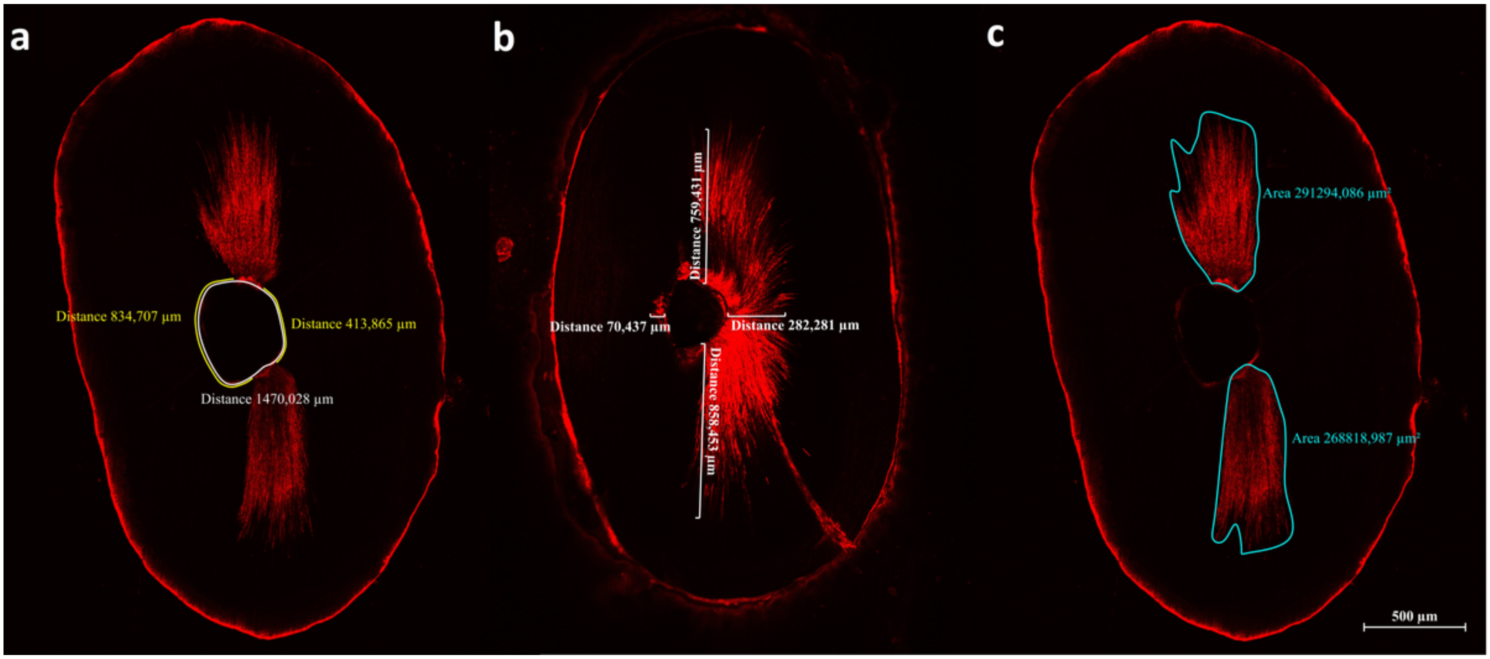

Figure 2

Image analyses procedures: [a] The non-penetration percentage, [b] maximum residual calcium hydroxide penetration depth and [c] penetration area of the calcium hydroxide inside the dentinal tubules.

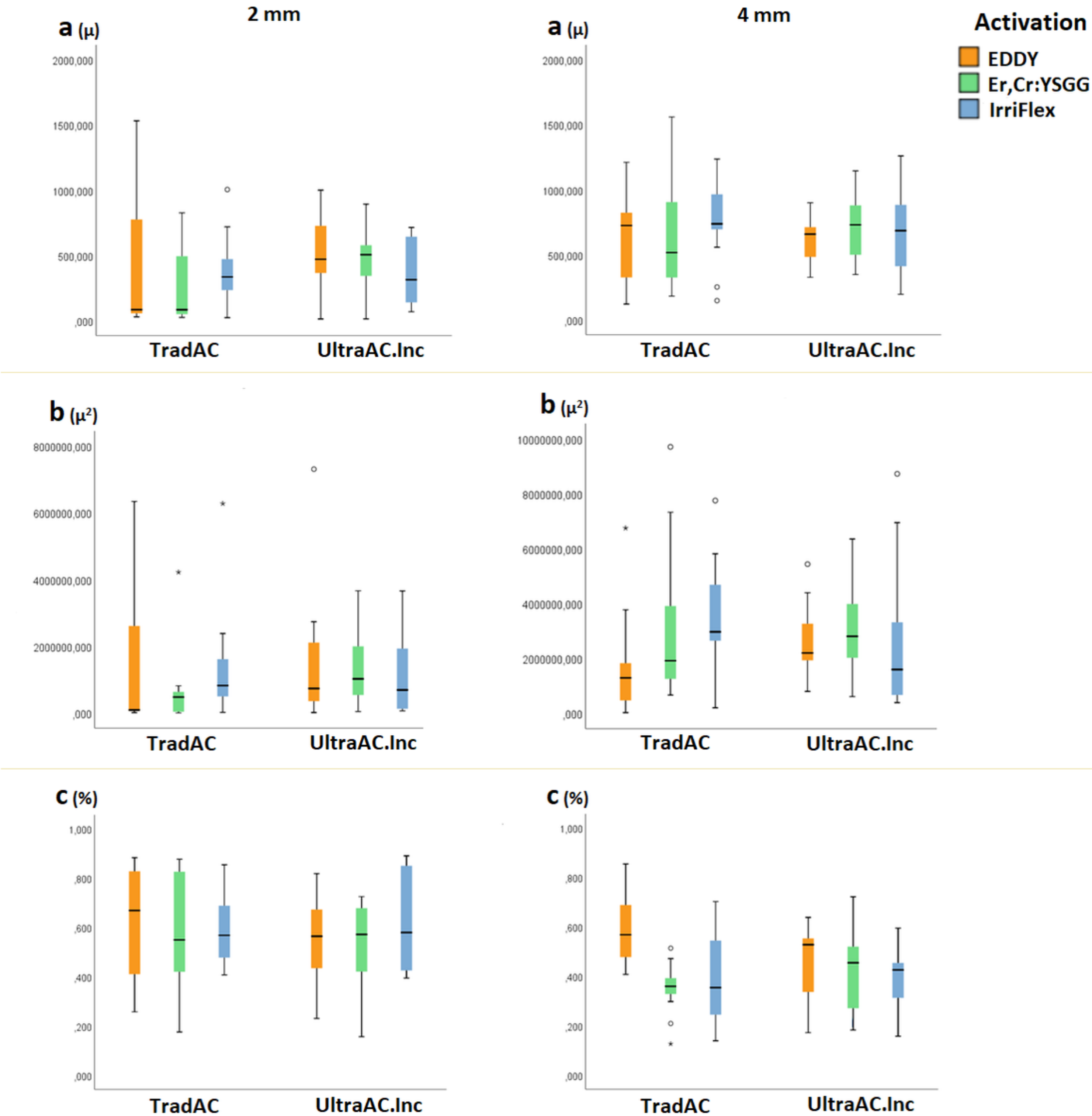

Figure 3

Box plot presentation of [a] penetration depth, [b] penetration area, and [c] non-penetration percentages measured at 2 and 4 mm levels from the apex for comparing TradAC and UltraAC.Inc with different cleaning protocols.

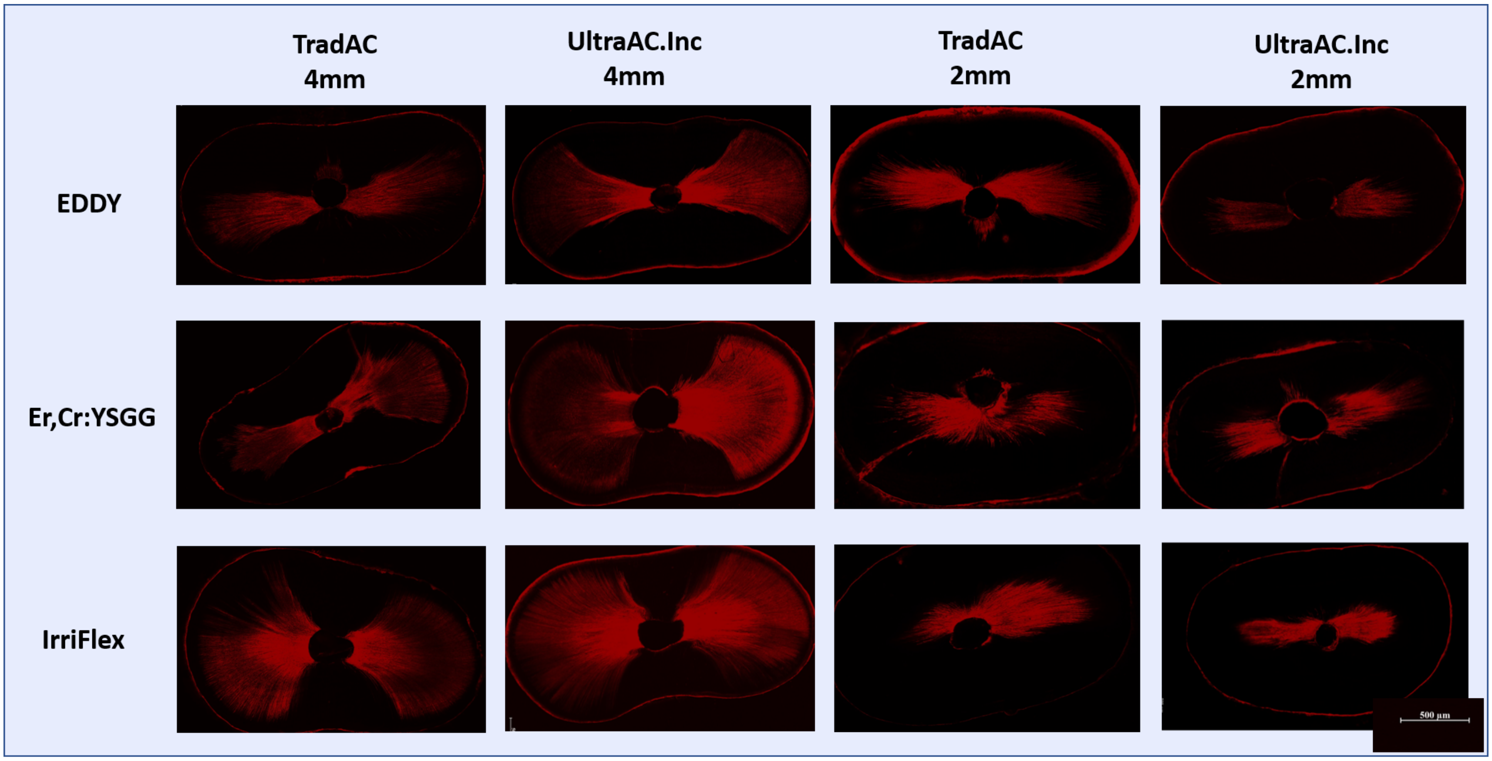

Figure 4

Confocal laser scanning microscopy representative images of the calcium hydroxide penetration of TradAC and UltraAC.Inc groups in the apical root third [4 and 2 mm] of specimens. The effect of access cavity preparation and cleaning protocols was not found statistically significant for both 2 and 4 mm level.