EGYPTIAN DENTAL JOURNAL
www.eda-egypt.org
VOL. 69, 3301:3308, OCTOBER, 2023
PRINT ISSN 0070-9484 • ONLINE ISSN 2090-2360
CONSERVATIVE DENTISTRY AND ENDODONTICS
Submit Date : 05-08-2023 • Accept Date : 15-08-2023 • Available online: 20-10-2023 • DOI : 10.21608/EDJ.2023.226799.2667

EFFECT OF DIFFERENT TAPERS AND ACTIVATION OF IRRIGATION ON DEPTH OF PENETRATION OF IRRIGANT USING CONFOCAL LASER MICROSCOPY (AN IN-VITRO STUDY)

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ABSTRACT

Aim: The aim of this in-vitro study was to evaluate the effect of different tapers on depth of penetration of irrigant using Confocal Laser Microscopy and weather the activation of irrigation could enhance deeper penetration of irrigant.

Materials and Methods: Fourty recently extracted permanent maxillary first molars were used in this study. Teeth were divided into 2 groups, 20 teeth in each group. Group (1): 4% taper and Group (2): 6% taper. Each group was subdivided into 2 subgroups, each containing 10 teeth. In Group (1), Subgroup (A): 4% taper without activation and Subgroup (B): 4% taper with activation. In Group (2), Subgroup (A): 6% taper without activation and Subgroup (B): 6% taper with activation. Access cavity was carried out in all teeth using round diamond bur. Mechanical preparation was performed using Edge File-X7 in mesio-buccal canal. The mesio-buccal root was separated from the tooth using a disc.Methylene blue dye (MB) was prepared using normal saline. In Subgroup (A) of both groups, MB dye was injected into the root canal using 3 mL syringe to stain the canal. The mesio-buccal root in both groups was then longitudinally split using a disc and mounted under Confocal Laser Microscopy. In Subgroup (B) of both groups, after injection of MB dye into root canal using 3 mL syringe, activation was performed, then the same procedures were carried out.

Results: Showed that 6% taper size significantly increased the penetration depth of the irrigant in both the non-activation and activation group compared to 4% taper size.

KEYWORDS: Syringe irrigation; Activation devices; Ultrasonic activation; Irrigation; Confocal laser microscopy.

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INTRODUCTION

For successful endodontic treatment, proper cleaning and shaping of root canal space is required. This can be fulfilled by the use of endodontic instruments and sufficient irrigation.[1] However, variation of root canal anatomy have influenced canal preparation to a great extent. Certain areas of root canal such as apical deltas, isthmuses and accessory canals cannot be reached with different instruments types.[2] Hence, for proper disinfection of root canal space, the use of chemical irrigation is mandatory.[3]

For chemical disinfection, different irrigating solutions have been introduced in endodontics. However, sodium hypochlorite (NaOCl) (Household cleaning products of Egypt) still remains the gold standard.[4,5] This is due to its dissolution effect on organic and necrotic tissues as well as its antimicrobial effect.[6] The combination of Ethylene Di-amide Tetra Acetic Acid (EDTA) and NaOCl for removing both organic and in-organic tissues is currently used as a final irrigation.[7]

Root canal space is small in its apical portion which make irrigating solutions difficult to contact canal walls in such area.[8] Hence, removal of smear layer and debris is less predictable compared to coronal and middle portions.[9] Bronnec et al[10] reported that shaping of root canals should be enlarged in-order to allow better flow of irrigating solutions. In contrast, for conservation of tooth structure, minimal canal enlargement apically has been suggested.[11]

There is a debate concerning the exact apical canal enlargement. It has been recommended at least three successive endodontic files should be used following the initial file for proper root canal enlargement.[12] Effect of different sizes and tapers on apical canal debridement have been carried out.[13,14,15]

There is increasing evidence that agitation of irrigant using different delivery systems like sonic activation, PIPS[3] and Ultra X Ultrasonic activator device (Eighteeth, Changzhou Sifary Medical Technology, China), better disinfect and clean root canal space when compared to conventional syringe irrigation technique which delivers the irrigant for only 1 mm deeper than the needle tip.[16]

Hence, the aim of this study was to evaluate the effect of different tapers on the volume of irrigant delivered at the working length in curved canals using Confocal Laser Microscopy (CLSM) and weather the activation of irrigation using Ultra X Ultrasonic activator device could enhance deeper penetration of irrigant.

MATERIALS AND METHODS

Fourty recently extracted permanent maxillary first molar with intact crowns and mesio-buccal root with 20-35 degree curvature were used in this study. Teeth were divided into 2 groups, each group contains 20 teeth. Group (1): 4% taper size and Group (2): 6% taper size. Each group was subdivided into 2 subgroups, each containing 10 teeth. In Group (1), Subgroup (A): 4% taper without activation and Subgroup (B): 4% taper with activation using Ultra X Ultrasonic activator device (Eighteeth, Changzhou Sifary Medical Technology, China). In Group (2), Subgroup (A): 6% taper without activation and Subgroup (B): 6% taper with activation using Ultra X Ultrasonic activator device (Eighteeth, Changzhou Sifary Medical Technology, China). The teeth were then kept in 0.9% saline solution at 4^0C for one day, then rinsed with distilled water. Samples were sterilized at 121°C for 20 minutes. All teeth were sealed at the apical third of the root to prevent the outflow of irrigating solution from the apical foramen.

For all teeth, access cavity was carried out using round diamond bur (Dentsply, Tulsa Dental, Dentsply Maillefer, USA) and mesio-buccal canal was established using K-file #10.

Mechanical preparation was performed using Edge File-X7 (by EDGE ENDO) in mesio-buccal canal. Teeth were enlarged to size 30/04 taper for Group (1) and to size 30/06 taper for Group (2). For

chemo-mechanical preparation, 5.25% NaOCl in a 3 mL syringe was used throughout the whole shaping procedure. EDTA gel (17%) (MD-Chelcream, Meta Biomed Co Ltd, Korea) was applied to each rotary file before being introduced in root canal. Teeth were then irrigated with a 2 mL/min of EDTA solution, then 3 mL/min of saline to stop the action of EDTA, then 3 mL/min NaOCl followed by 5% sodium thiosulphate to inactivate NaOCl and a final flush with 3 mL/min saline to remove any remaining solution using 3 mL syringe shorter than the working length by 1 mm. The mesio-buccal root was separated from the tooth using a disc and standardized to 16 mm working length.

Methylene blue dye (MB) with wavelength 660-665 nm, at concentration of 0.5 mg/mL was prepared using normal saline. In Subgroup (A) (without activation) in both groups, MB dye was injected into the root canal using 3 mL syringe to stain the canal from the coronal to apical part. The mesio-buccal root in both groups was then longitudinally split using a disc. The sections were polished with silicon abrasive carbide paper to remove debris which could have been created during the sectioning procedures. The sections were then mounted on glass slides and examined under Confocal Laser Microscopy (CLSM) (National Research Centre, Dokki, Egypt) with a dry lens (0.3 numeric aperture) and X10 magnification to figure out the depth of irrigant penetration **Fig (1) and (2).**

In Subgroup (B) (with activation) in both groups, after injection of MB dye into root canal using 3 mL syringe, activation was performed using Ultra X Ultrasonic activation device, then the same procedures were carried out **Fig (3) and (4).**

Statistical analysis:

Numerical data was represented as mean and standard deviation (SD) values. Shapiro-Wilk's test was used to test for normality. Homogeneity of variances was tested using Levene's test. Data showed parametric distribution and variance homogeneity and were analyzed using two-way ANOVA. Comparison of simple main effects was done utilizing the error term of the two-way model with p-values adjustment using Bonferroni correction. The sig-

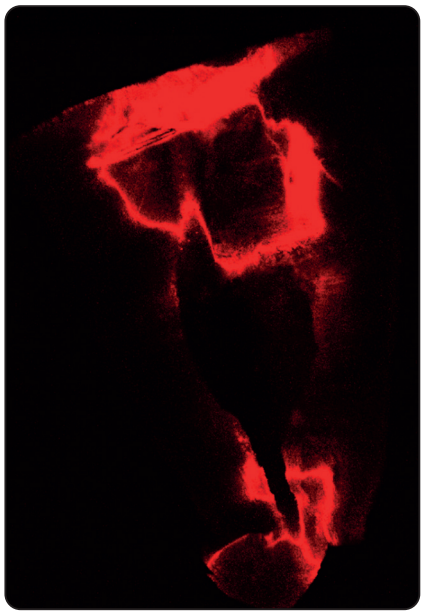

Fig. (1): Image showing depth of penetration of irrigant in Group (1), Subgroup (A) (4% taper, without activation)

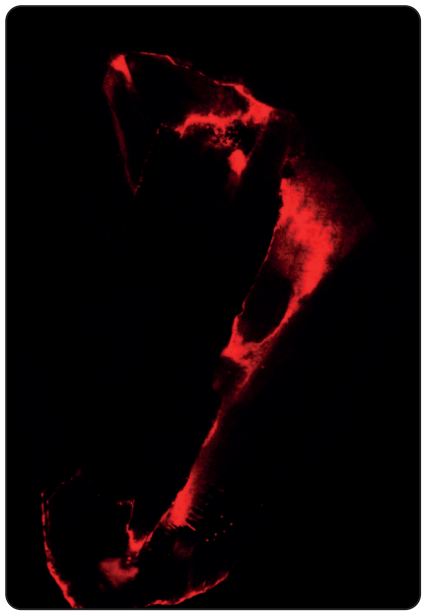

Fig. (2): Image showing depth of penetration of irrigant in Group (2), Subgroup (A) (6% taper, without activation)

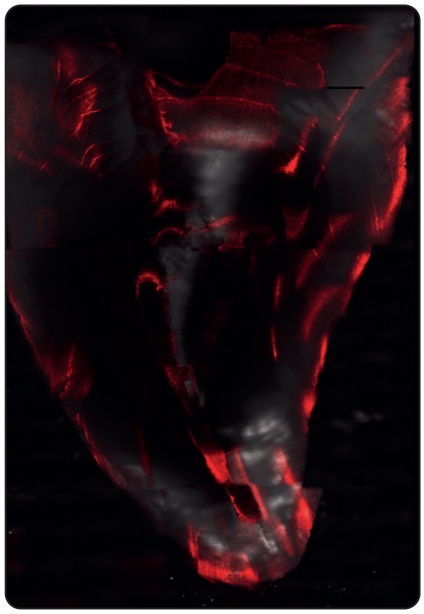

Fig. (3) Image showing depth of penetration of irrigant in Group (1), Subgroup (B) (4% taper, with activation)

Fig. (4) Image showing depth of penetration of irrigant in Group (2), Subgroup (B) (6% taper, with activation)

nificance level was set at $p<0.05$ within all tests. Statistical analysis was performed with R statistical analysis software version 4.3.0 for Windows*.

RESULTS

Results of two-way ANOVA are presented in table (1). They showed that there was a significant interaction effect between file taper and activation on penetration depth ($p<0.001$). Comparison of simple main effects are presented in table (2). Within activated and non-activated samples, files with taper (6%) had significantly higher penetration depths ($p<0.001$). For taper (4%), activation had no significant effect ($p=0.088$), while for taper (6%) samples, significantly higher penetration was achieved with activation ($p<0.001$). Mean and standard deviation values for penetration depth in different variables are presented in figures (5) and (6).

TABLE (1) Two-way ANOVA test results

Parameter	*Sum of squares*	*df*	*Mean square*	*f-value*	*p-value*
Taper	6879.48	1	6879.48	220.96	**<0.001***
Activation	420.86	1	420.86	13.52	**<0.001***
Taper* activation	1171.07	1	1171.07	37.61	**<0.001***

significant (p<0.05)

TABLE (2) Comparisons of simple main effects

Activation	*Penetration depth (pixels) (Mean±SD)*		*f-value*	*p-value*
	Taper (4%)	*Taper (6%)*		
Activated	1047.62±4.59	1075.28±5.57	**245.79**	**<0.001***
Non activated	1051.37±5.03	1060.29±7.64	**12.78**	**<0.001***
f-value	**3.02**	**48.11**		
p-value	**0.088**	**<0.001***		

significant (p<0.05)

* R Core Team (2023). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

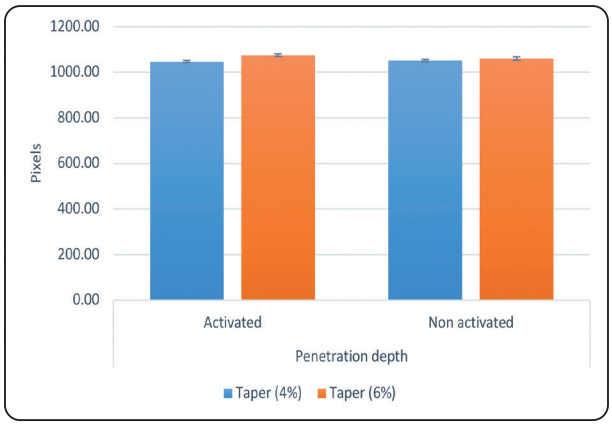

Fig. (5) Bar chart showing mean and standard deviation values of penetration depth (pixels)

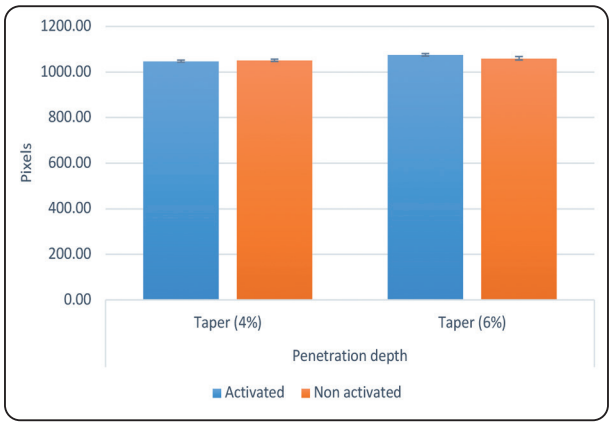

Fig. (6) Bar chart showing mean and standard deviation values of penetration depth (pixels)

DISCUSSION

During root canal treatment, it is very important, besides shaping and cleaning procedures, that the root canal be chemically disinfected.[17] Although, endodontic files weather manual or rotary have been improved, 40% of the root canal following mechanical procedures may remain intact.[18]

Therefore, for proper bacterial and smear layer elimination, Giardino et al [19] and Soares et al [20] recommended the use of 17% EDTA followed by 5.25% NaOCl. Hence, 17% EDTA and 5.25% NaOCl were used in this study.

Canal preparation is influenced by curvature, hence, the following study was performed on curved mesio-buccal canals of permanent maxillary first molars ranging from 20-35 degree curvature, like some other studies.[21,22]

For better removal of contents from the root canal space, it was recommended that the irrigating needle should be placed at the working length or 1 mm shorter than the working length.[23] Therefore, for proper irrigation, in this study, the needle was placed 1 mm shorter than the working length. In order to place the needle as close as possible to the apical third of the canal, it is necessary to increase the root canal preparation size and taper.[24]

Taper is a main factor that can determine the root canal dimensions which can affect the action of irrigants.[13] Therefore, the objective of the following study was to evaluate the influence of different tapers on the volume of irrigant delivered at the working length. In order to achieve this, the apical canal size was standardized to file size 30, with two different tapers. Therefore, Edge file X7 rotary file was used in this study as it has a constant taper of 4% or 6% at the apical and middle thirds of the canals.

Although the use of syringe and needle to irrigate the root canal is the most commonly used method, it didn't guarantee proper cleaning of the root canal space.[25] Curved canals present a challenge to clean and disinfect the root canal due to the contact points that occur between the needle and canal walls, therefore, bending of the instrument is necessary to follow the canal curvature. This could result in compromising the irrigant efficiency and a risk of canal transportation.[25] Such challenge could be overcomed by the use of activation techniques as reported by Malki et al [26], Macedo et al [27] and Al-Jadaa et al [28], who stated that Passive Ultrasonic Irrigation (PUI) improved the efficacy of irrigating solution in curved canals. Therefore, Ultra X Ultrasonic activation device was used in the following study to figure out if activation of the irrigant will enhance deeper penetration of the irrigant in both taper 4% and 6% compared to syringe irrigation technique.

Methylene blue dye (MB) was used in this study to assess the penetration depth of NaOCl as some pilot studies exhibited advantages to MB dye compared to other dyes as dentine shows auto-fluorescence.[29] MB dye showed proper penetration depth which comes in line with other studies, even superior penetration depths from the apex.[29, 30]

Confocal Laser Microscopy (CLSM) gives an accurate information about the depth and distribution of the irrigant inside the root canal space where multiple sections could be taken and reconstructed to produce a final image.[31] For this reason, CLSM was used in this study.

It was found out that increasing the preparation size of the apical foramen more than #30 enhance better removal of debris and efficiently clean the apical third of the root canals. But, unfortunately, these sizes could lead to ledges, canal transportation, and perforations especially in curved narrow canals.[32, 33] Hence, the apical preparation size was standardized to #30 for all teeth due to the use of narrow curved canals in this study.

Minimal preparation size and taper especially when using an activation device like Ultra X Ultrasonic device will enhance proper penetration of irrigant while conserving the tooth structure and avoiding its weakening especially at the cervical area of the tooth. In this study, 4% taper size resulted in deeper penetration of irrigant when activation device was used compared to non-activation, but the result wasn't statistically significant. This came in agreement with Brunson et al,[34] who stated that 4% taper was adequate for efficient volume of irrigant when using EndoVac irrigation system. In 6% taper size, the activation device resulted in significantly higher penetration depth compared to non-activation. This could be attributed to the fact that activation devices produce acoustic micro-streaming which efficiently activates the irrigant allowing its deeper penetration and proper cleaning of the root canal space.[35]

In the present study, the results also showed that 6% taper size significantly increased depth of penetration of irrigant in both non-activated and activated groups compared to 4% taper size. This could be attributed to the fact that increasing the taper size allow for better introduction of the irrigant while keeping the apical size small.[18] This came in agreement with Khademi et al,[14] who reported that using a 6% taper size file enhanced deeper penetration of the irrigant and improved the replacement of the irrigant compared to 4% taper size. Also, similar results were seen in other in-vitro studies who reported that increasing the taper size from 4% to 6% allow deeper penetration of the irrigant.[4, 18]

CONCLUSION

Within the limitation of this study, it could be concluded that increasing the taper size in mesio-buccal canal of permanent maxillary first molar from 4% to 6% significantly allowed deeper penetration of the irrigant in both non-activation and activation groups.

Therefore, an apical preparation size #30 with 6% taper allowed for deeper irrigant penetration in apical third of the root canal, with Ultra X Ultrasonic activator device performing much better than with non-activation.

Further studies are needed to assess the exact apical size and taper which allows deeper penetration of irrigants within the root canal space while conserving the tooth structure.

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