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ASSESSMENT OF SEALER PENETRATION OF BIOCERAMIC SEALER WITH DIFFERENT ACTIVATION TECHNIQUES USING CONFOCAL LASER MICROSCOPY (AN IN-VITRO STUDY)

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ABSTRACT

Aim: To assess the effect of different activation techniques on the area of sealer penetration after using different sealers and different obturating materials.

Materials and Methods: Forty single rooted mandibular premolars are collected and were prepared with Protaper next up to size x4. Teeth were divided into 2 groups according to activation technique, each containing 20 teeth. Group (1): manual activation, Group (2): laser activation. Each group was subdivided into 2 groups according to sealer and obturating material, each containing 10 teeth. Subgroup (A): teeth were obturated with Adseal resin sealer mixed with methylene blue dye and Gutta Percha points, Subgroup (B): teeth were obturated with Ceraseal Bioceramic sealer mixed with methylene blue dye and C-Points. The teeth were sectioned at 3 mm and 6 mm from the apex and examined with Confocal Laser Scanning Microscopy.

Results: Diode laser activation and Bioceramic sealer+C-Points have significantly higher sealer penetration area than manual activation and Adseal resin sealer+Gutta percha points.

Conclusion: Diode laser activation has significantly more area of sealer penetration than Manual activation method. Also, Bioceramic sealer+C-Point has significantly higher area of sealer penetration compared to Adseal resin sealer+Gutta Percha points.

KEYWORDS : Diode laser, Bioceramic sealer, C-Point, Irrigant activation, Confocal Laser Scanning Microscopy

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INTRODUCTION

Eradication of infection in the pulp space is the main reason for successful endodontic treatment. This could be accomplished through appropriate cleaning and shaping of the root canal space and using a biocompatible obturation material to fill such space.[1]

For effective endodontic treatment, proper irrigation should be performed as it attains a lot of chemical, mechanical and biological functions.[2]The main goal of irrigation is the removal of microorganisms and debris during root canal instrumentation, disrupt the bacterial biofilms and breaking down necrotic pulp tissue.[3]

Several irrigations have been used in endodontics. However, sodium hypochlorite (NaOCl) is the most commonly used irrigant in root canal treatment as it is capable of dissolving organic debris and removing necrotic tissues along with biofilm.[2]Smear layer removal through irrigation has been observed in many root canal irrigation studies. It can be removed through the combination of sodium hypochlorite (NaOCl) and Ethylene Diamine Tetra Acetic Acid (EDTA) as this combination dissolves both the organic and inorganic contents of the smear layer.[4] However, irrigation alone failed to completely remove the smear layer.

Several methods and a lot of devices have been used for proper disinfection of the root canal and complete eradication of the smear layer.[5] Lasers have become an era for improving the efficiency of irrigating solutions. Diode laser is efficient in removing the smear layer and have a wavelength similar to the infrared range, so, it has been recommended to be used in endodontics.[6]

Root canal filling is another important factor for successful endodontic treatment. Gutta-percha (GP) and root canal sealers are the current accepted obturation methods. However, it has no adhesive criteria to dentin regardless to the obturation technique used.[7] Root canal sealer should properly seal the root canal space and penetrate small inaccessible areas (dentinal tubules) during compaction providing proper adhesion to dentin.[8]

Bioceramic sealers have become popular recently. Calcium silicate-based sealers are radiopaque and hydrophilic, hence creating hydroxyapatite when setting and adhering properly to root canal dentin as it demonstrates no shrinkage and little expansion while setting.[1]

Hence, this study aimed to evaluate the effect of diode laser activation on area of penetration of sealer after using different obturating materials and sealers. The null hypothesis was that either activation with diode laser or not would affect the area of sealer penetration of teeth filled with two different obturating materials.

MATERIALS AND METHODS

Sample selection

Forty single rooted mandibular premolar teeth with mature apices and root canal curvature ≤5°, calculated using the method of Scheider were included in this study. Any teeth with immature roots, internal or external resorption, root cracks or previous endodontic treatment were excluded. Teeth were disinfected by being immersed in 5.25% sodium hypochlorite (NaOCl) for 30 minutes, then stored in 0.9% saline solution till usage.

Sample preparation

All teeth were then de-coronated using a low speed diamond disc under sufficient coolant and tooth length was adjusted to be 16 mm from anatomic apex using a k-file size #10 (MANI, Inc, Utsunomiya, Tochigi, Japan). Root canal preparation was carried out using Protaper Next rotary system (Dentsply Maillefer, Ballaigues, Switzerland) till file size X4 (#40/0.06) with speed and torque adjusted according to manufacturer's instructions with an electric motor (X Smart; Dentsply, Maillefer, Ballaigues, Switzerland). Irrigation was performed using 3 ml of 2.5% NaOCl after each file and 17% Ethylene Diamine Tetra Acetic Acid gel (EDTA) (MD-Chelcream, Meta Biomed Co Ltd, Korea).

Teeth were then divided into two main groups, each containing 20 teeth, according to the last irrigation technique:

Group (1): Manual activation technique where teeth were filled with 5 ml of 2.5% NaOCl and push-pull strokes were done manually up to the working length using a #40 gutta-percha cone approximately at a rate of 100 strokes per minute for 30 seconds. The canal was irrigated again with 2.5 ml of 2.5% NaOCl and the same procedure was repeated for another 30 seconds. This cycle was repeated using 5 ml of 17% EDTA solution . Hence, each irrigant was activated for 1 minute with a total volume of 5 ml per irrigant. 5 ml distilled water was used between NaOCl and EDTA and as a final flush.

Group (2): Diode laser activation (940 nm) technique. Biolase Epic XTM (Biolase, Irvine, California, USA) was applied using a tip number E2-14 (Biolase, Irvine, California, USA); endo 200-µm malleable laser tip and a length of 14 mm, being short of the apex by 1 mm, with wave length 940nm ± 10nm with consistent settings of 2 watt. The laser tip was detached in gentle, helical movements, in an apical-coronal path to guarantee even light diffusion inside the root canal wall at a speed of about 2 mm/sec. The irrigation/activation protocol was as follows: 1.25 ml 2.5% NaOCl for 5 seconds time periods, then diode laser activation for another 5 seconds. This lasing cycle was repeated for 4 times. Radiation lasted for a total of 20 seconds. After rinsing the canals with 2.5 ml distilled water (DW), same protocol of irradiation was applied with the 17% EDTA. Thus, 1.25 mL of EDTA was used at each lasing cycle and the procedure was repeated four times. Consequently, the total radiation exposure for both irrigants was 40 seconds. Finally, the canals were rinsed with 2.5 mL distilled water.[9]

Teeth in each group were further divided into two subgroups (n=10) according to the type of obturating material and sealer used:

Subgroup (A): Teeth were obturated using single gutta-percha (GP) cone size X4 (Dentsply Maillefer, Ballaigues, Switzerland) and Adseal resin sealer (Dentsply, Konstanz, Germany) where the sealer was mixed with methylene blue dye (MB) with wavelength 660-665 nm before obturation.

Subgroup (B): Teeth were obturated using single C-point cone size F4 (Endo Technologies, LLC, Shrewsbury, MA, USA) and Ceraselal bioceramic sealer (Meta-Biomed, Cheongju, Republic of Korea) where the sealer was mixed with methylene blue dye (MB) with wavelength 660-665 nm before obturation.

For complete setting of sealer, teeth were then stored at 37°C with 100% moisture for two weeks.

Sample sectioning

Roots were sectioned using a slow speed diamond disc under water coolant at distances 3 and 6 mm from the apex to provide the middle and apical sections of the root. Root sections were then mounted on glass slides and examined under Confocal Laser Scanning Microscope (CLSM) (National Research Centre, Dokki, Egypt) with a dry lens (0.3 numeric aperture) and X10 magnification. **Fig. (1), (2), (3) and (4).**

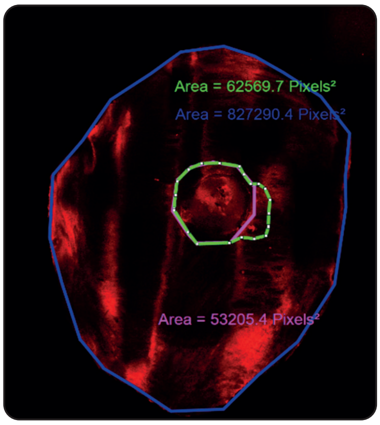

Fig. (1) Image showing area of sealer penetration in middle portion of root in Group (2), Subgroup (A) (GP+Adseal resin sealer with Diode laser activation)

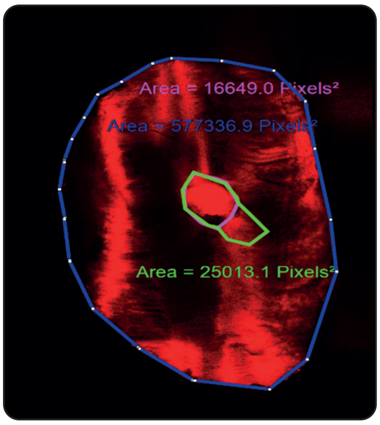

Fig. (2) Image showing area of sealer penetration in apical portion of root in Group (2), Subgroup (A) (GP+Adseal resin sealer with Diode laser activation)

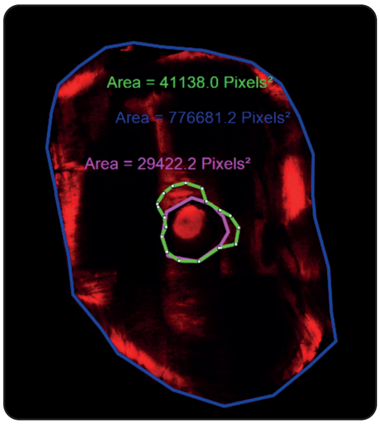

Fig. (3) Image showing area of sealer penetration in middle portion of root in Group (2), Subgroup (B) (C-point + Ceraseal Bioceramic sealer with Diode laser activation)

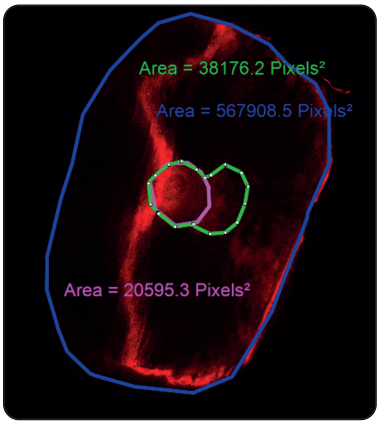

Fig. (4) Image showing area of sealer penetration in apical portion of root in Group (2), Subgroup (B) (C-point + Ceraseal Bioceramic sealer with Diode laser activation)

Statistical analysis

Numerical data was represented as mean and standard deviation (SD) values. The normality of the data was inspected by viewing the data distribution and using Shapiro-Wilk's test. The variance homogeneity and sphericity assumptions were confirmed using Levene's and Mauchly's tests, respectively. The data were analyzed using a three-way mixed model ANOVA. Comparisons of simple effects were made utilizing the error term of the three-way model with p-values adjustment using the False Discovery Rate (FDR) method. The significance level was set at p<0.05 within all tests. Statistical analysis was performed with R statistical analysis software version 4.3.3 for Windows*.

RESULTS

Results of the three-way ANOVA presented in Table (1) showed that there was a significant interaction effect between the tested variables (p<0.001).

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* R Core Team (2024). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Comparisons of simple effects presented in Tables (2) and (3) showed that regardless of the obturation technique and the root section, the area of penetration measured with laser activation was significantly higher than that achieved with manual activation ($p<0.001$). Additionally, they showed that regardless of activation method and root section, samples obturated using C-point and bioceramic sealer had significantly higher penetration than those obturated using GP and resin sealer ($p<0.001$). Finally, they showed that penetration measured in the apical sections was significantly higher than that measured in the middle sections ($p<0.001$). Mean and standard deviation values for trueness (RMS) for different variables are presented in Figures from (5) to (7).

TABLE (1) Three-way ANOVA test results.

Parameter	*Sum of squares (II)*	*df*	*Mean square*	*f-value*	*p-value*
Activation method	454266524.45	1	454266524.45	**6267.88**	**<0.001***
Obturation technique	158523912.45	1	158523912.45	**2187.28**	**<0.001***
Root section	98302666.80	1	98302666.80	**1437.49**	**<0.001***
Activation* obturation	67467501.11	1	67467501.11	**930.90**	**<0.001***
Activation* section	17135262.56	1	17135262.56	**250.57**	**<0.001***
Obturation* section	22477724.36	1	22477724.36	**328.69**	**<0.001***
Activation* obturation* section	21794126.64	1	21794126.64	**318.70**	**<0.001***

****Significant (p<0.05).***

TABLE (2) Summary statistics and simple main effects.

Root section	*Obturation technique*	*Area of penetration (pixel²) (Mean±SD)*		*f-value*	*p-value*
		Manual activation	*Laser activation*		
Middle	***GP and resin sealer***	6167.91±265.11	9215.36±195.93	**659.30**	**<0.001***
	C-point and bioceramic sealer	7130.34±72.33	11763.36±144.45	**1523.84**	**<0.001***
	f-value	**65.76**	**460.90**		
	p-value	**<0.001***	**<0.001***		
Apical	***GP and resin sealer***	7443.06±173.82	10253.96±398.35	**560.92**	**<0.001***
	C-point and bioceramic sealer	8437.98±80.04	17010.01±483.08	**5216.50**	**<0.001***
	f-value	**70.27**	**3240.39**		
	p-value	**<0.001***	**<0.001***		

****Significant (p<0.05)***

TABLE (3) Summary statistics and simple main effects.

Activation	*Obturation technique*	*Area of penetration (pixel²) (Mean±SD)*		*f-value*	*p-value*
		Middle section	*Apical section*		
Manual	***GP and resin sealer***	6167.91±265.11	7443.06±173.82	**118.87**	**<0.001***
	C-point and bioceramic sealer	7130.34±72.33	8437.98±80.04	**125.02**	**<0.001***
Laser	***GP and resin sealer***	9215.36±195.93	10253.96±398.35	**78.87**	**<0.001***
	C-point and bioceramic sealer	11763.36±144.45	17010.01±483.08	**2021.67**	**<0.001***

Significant (p<0.05)

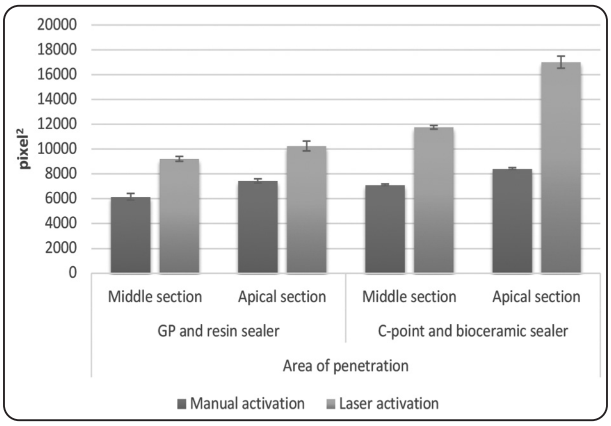

Fig. (5) Bar chart showing mean and standard deviation values of the area of penetration (A).

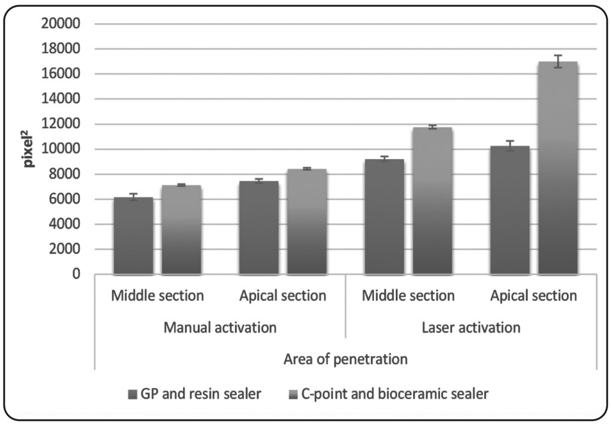

Fig. (6) Bar chart showing mean and standard deviation values of the area of penetration (B).

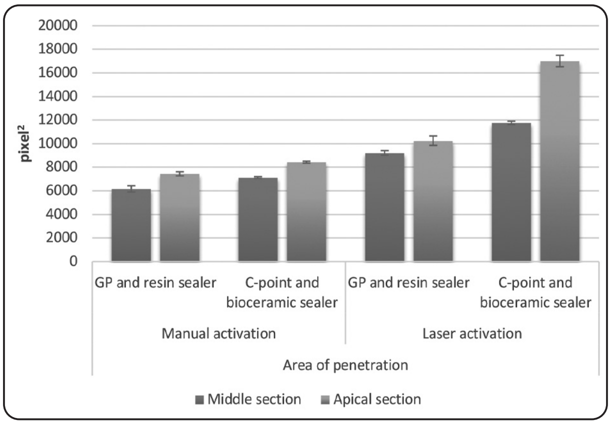

Fig. (7) Bar chart showing mean and standard deviation values of the area of penetration (C).

DISCUSSION

The main aim of endodontic treatment is to hermetically fill the root canal space three-dimensionally, following the effective removal of pulp tissues and smear layer formed during chemo-mechanical preparation of the root canal.[10]

One of the main cause of pulp and peri-apical infections is bacteria. Hence, for proper elimination of such bacteria and better disinfection, it has been insinuated to use different activation techniques.[11]

Several studies have examined the penetration of sealer in dentinal tubules.[12,13] Some studies examined the depth of sealer penetration after applying different irrigation activation techniques[14,15], while other studies only examined depth of penetration of sealer.[16,17]

Therefore, this study aimed to evaluate the depth of penetration of two root canal filling materials following manual activation and laser activation of irrigating solution using CLSM.

Single-rooted mandibular premolar teeth were used in the following study, owing to their oval cross-section which can't be properly cleaned and shaped with the round cross-section design of most endodontic files. Hence, irrigant activation is essential in such canals for proper disinfection and adhesion of root canal filling material.[18]

ProTaper Next rotary instrument was used in this study as it is flexible, simple, efficient, safe and gives a more uniform, centered and rounded prepared canal.[19]

NaOCl and EDTA with different activation methods were used in this study for irrigating the root canal space in order to remove the smear layer effectively, thus ensuring proper penetration of the root canal sealer and increasing the sealing ability.[20]

Diode laser was used in this study for activation of root canal irrigant and removal of smear layer, as it has an excellent depth of penetration into dentinal tubules (500 μm) compared to chemical solutions (100 μm).[21] Also it has an excellent antimicrobial effect, safe wavelength, low cost and low temperature rise.[22]

In the current study, Bioceramic sealer was used for obturating the root canal space owing to its hydrophilic nature. It was used with C-Point as the bioceramic particles in the sealer bond to the outer surface of C-Points in addition to bonding to dentin by formation of hydroxyapatite. C-Points consists of a central core which is coated by a hydrophilic polymer that expands allowing sealer penetration into lateral canals and dentinal tubules, hence, improving seal and adhesion.[23]

CLSM was used in this study owing to its non-destructive technique and less artifacts. It allows taking photos from different depths which can then be merged to create a final image.[24] Also, it has smaller magnification sizes where a whole sample surface can be examined.[13]

In this study, methylene blue dye (MB) was used to evaluate the depth of sealer penetration into dentinal tubules, as it has superior depth of penetration from the apex.[25]

Sectioning of samples was taken at 3 and 6 mm from the apex in this study, in order to overcome the presence of apical ramifications and anatomical irregularities .[26]

In the present study, the area of penetration was assessed by drawing the area around the areas where the sealer penetrated and then subtracting the area which is measured by drawing the circumference of the canal.[27]

Results in this study showed that Diode laser activation significantly increased area of penetration of sealer into dentinal tubules compared to manual activation technique, regardless the obturating material used and root section. This comes in agreement with Kundabala et al [28] who concluded that diode laser has better performance when compared to other irrigant combinations. The reason behind this better performance might be due to its mode of delivery, (500 μm) fiber optic tip with 14 mm length.[28] Also, it allows proper removal of smear layer.[29]

In the present study, results also showed that Bioceramic sealer with C-Points significantly increased area of sealer penetration compared to Adseal resin sealer with GP points regardless the activation method used and root section. This came in agreement with Hachem et al.[30], Candeiro et al.[31] and Wang et al.[32] who stated that bioceramic sealer has a significantly greater depth of penetration compared to resin sealer. This might be due to the size of the particles which is smaller in bioceramic sealer (2mm) compared to resin sealer (8 mm) where smaller particle size allows more penetration into dentinal tubules and act as a physical barrier preventing micro-leakage.[30] Also, hydrophilic sealers have more penetration depth than hydrophobic sealers.[33] In addition, C-Point expands due to its hydrophilic nature which allow more penetration of sealer into dentinal tubules, increasing adhesion and seal.[23]

Finally, results in this study showed that apical sections (at 3 mm) have a significantly higher area of sealer penetration compared to middle sections (at 6 mm). This could be explained according to Giudice et al.[34] who found that the average number of dentinal tubules in apical area is similar to coronal area, while being lowest in number in the middle area. This finding supports the results of Gaston et al.[35] who found that the bond strength in apical area was significantly higher than coronal and middle areas.

CONCLUSION

From the following study, it could be concluded that Diode laser activation and Bioceramic sealer+C-Point significantly increased area of sealer penetration compared to manual activation and Adseal resin sealer+GP, regardless the obturating material used and root section. In addition, apical sections have significantly higher area of sealer penetration than middle sections.

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