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## Impact of Contracted Endodontic Access Cavity Designs on Root Canal Disinfection Using Diode Laser: An in-Vitro Study

Mohamed M.N. El-Tayeb Department of Endodontics, Faculty of Oral and Dental Medicine, Badr University in Cairo, Cairo, Egypt

Mohamed Nabeel Endodontic Department, Faculty of Oral and Dental Medicine, Misr International University, Cairo, Egypt

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## Endodontics / Endodontie

## IMPACT OF CONTRACTED ENDODONTIC ACCESS CAVITY DESIGNS ON ROOT CANAL DISINFECTION USING DIODE LASER: AN *IN-VITRO* STUDY

Mohamed M.N. El-Tayeb<sup>1</sup> | Mohamed Nabeel<sup>2</sup>

**Introduction**: New approaches for minimal invasive endodontic access cavity designs had been introduced for preserving the structural integrity of the offending tooth. Diode laser has been now widely used in root canal disinfection and showing highly promising results.

**Aim**: to evaluate the impact of contracted endodontic access cavity designs on root canal disinfection using diode laser.

**Materials and Methods**: Fifty-four intact freshly extracted human mandibular first molars were selected for the use in this study. Only the mesial roots of the selected samples were included in this study. Samples were randomly divided into three equal groups (n=18) according to the type of access cavity performed. Group (1): conventional access cavities, Group (2): Ninja access cavities, and Group (3): truss access cavities. Isolates of Enterococcus Faecalis (E.Fecacalis) were introduced in the root canals of the mesial roots of the selected samples. Samples were further sub-divided into two equal sub-groups (n=9) according to the disinfection method. Sub-group (a): Diode laser disinfection, and Sub-group (b): NaOCI 2.5% followed by EDTA 17% irrigation. Bacterial evaluation was performed using confocal laser scanning electron microscope.

**Results**: The results showed that using Diode laser disinfection have shown higher bacterial reduction in comparison to NaOCI irrigation, but with no statistically significant difference. For access cavity designs, regardless of the type of disinfection used, conventional access cavities have shown the highest bacterial reduction, with the least bacterial reduction with Ninja access cavities.

**Conclusion**: Contracted endodontic access cavities did not offer any advantages in comparison with the conventional endodontic access cavities regarding bacterial reduction. Diode laser can be used as an effective adjunct tool in root canal disinfection.

**Keywords**: Conventional endodontic access cavities, Minimal endodontic access cavities, Sodium hypochlorite irrigation, Diode laser, Confocal Laser Scanning Microscope.

#### Correspondence to:

Mohamed M.N. El-Tayeb. E-mail: eltayeb65@hotmail.com

#### **Conflicts of interest:**

The authors declare no conflicts of interest.

- 1- Lecturer of Endodontics, Department of Endodontics, Faculty of Oral and Dental Medicine, Badr University in Cairo, Cairo, Egypt. eltayeb65@hotmail.com
- 2- Lecturer of Endodontics, Endodontic Department, Faculty of Oral and Dental Medicine, Misr International University, Cairo, Egypt. dr.neebo@hotmail.com

### **ORIGINAL ARTICLE** / ARTICLE ORIGINAL

## Endodontics / Endodontie

## IMPACT DU DESIGN DES CAVITÉS D'ACCÈS ENDODONTIQUES CONTRACTÉES SUR LA DÉSINFECTION DU CANAL RADICULAIRE À L'AIDE D'UN LASER À DIODE : UNE ÉTUDE IN VIVO

**Introduction**: De nouvelles approches pour le design de cavités d'accès endodontiques mini-invasives ont été introduites pour préserver l'intégrité structurelle de la dent impliquée. Le laser à diode est désormais largement utilisé dans la désinfection des canaux radiculaires et donne des résultats très prometteurs.

**Objectif**: évaluer l'impact du design des cavités d'accès endodontiques contractées sur la désinfection canalaire à l'aide d'un laser à diode.

Matériels et méthodes: Cinquante-quatre premières molaires mandibulaires humaines intactes et fraîchement extraites ont été sélectionnées pour être utilisées dans cette étude. Seules les racines mésiales des échantillons sélectionnés ont été incluses dans cette étude. Les échantillons ont été répartis au hasard en trois groupes égaux (n = 18) en fonction du type de cavité d'accès réalisée. Groupe (1) : cavités d'accès conventionnelles, Groupe (2) : cavités d'accès Ninja et Groupe (3) : cavités d'accès en treillis. Des isolats d'Enterococcus Faecalis (E.Fecacalis) ont été introduits dans les canaux radiculaires des racines mésiales des échantillons sélectionnés. Les échantillons ont ensuite été subdivisés en deux sous-groupes égaux (n = 9) selon la méthode de désinfection. Sous-groupe (a) : Désinfection laser à diode, et Sous-groupe (b) : NaOCl 2,5% suivi d'une irrigation EDTA 17%. L'évaluation bactérienne a été réalisée à l'aide d'un microscope électronique confocal à balayage laser. Résultats : Les résultats ont montré que l'utilisation de la désinfection au laser à diode a montré une réduction bactérienne plus élevée que l'irrigation au NaOCI, mais sans différence statistiquement significative. Pour les conceptions de cavités d'accès, quel que soit le type de désinfection utilisé, les cavités d'accès conventionnelles ont montré la réduction bactérienne la plus élevée, avec la réduction bactérienne la plus faible avec les cavités d'accès Ninja. Conclusion : Les cavités d'accès endodontiques contractées n'offraient aucun avantage par rapport aux cavités d'accès endodontiques conventionnelles en termes de réduction bactérienne. Le laser à diode peut être utilisé comme outil complémentaire efficace dans la désinfection du canal radiculaire.

**Mots clés** : Cavités d'accès endodontiques conventionnelles, Cavités d'accès endodontiques minimales, Irrigation à l'hypochlorite de sodium, Laser à diode, Microscope confocal à balayage laser.

### Introduction

The goal of root canal treatment is to eliminate harmful pathogens from the root canal and create a hostile environment in which no organism can survive. Proper cleaning and shaping, effective irrigation and disinfection, and three-dimensional obturation to seal the root canal are all necessary for an endodontic treatment to be successful. A precise access cavity preparation is all that is needed for successful endodontic treatment to achieve these goals [1, 2].

During conventional access cavity preparation, additional tooth structure will be sacrificed, which may ultimately reduce the tooth's fracture resistance. The amount of remaining dentin is the most important factor in determining the fracture resistance and longevity of endodontically treated teeth. Because of the removal of internal tooth structure during endodontic therapy, endodontically treated teeth are more likely to fracture than sound teeth [3, 4].

Minimally invasive dentistry and new concepts of conservative endodontic access cavity designs have been introduced to avoid the challenges of unnecessary tooth structure loss. The idea is to preserve and maintain as much sound dentin as possible, which is crucial for increasing the survival and longevity of the teeth [5, 6].

However, there is still a clinical dilemma regarding the impact of conservative access designs in effective elimination of bacterial load in infected root canals. Since such designs might hinder efficient instrumentation and proper irrigation during endodontic root canal therapy. Thus, new methods and innovative techniques have started to gain popularity for enhancing root canal disinfection, the cleaning of the endodontic space, and debris removal, which are prerequisites for improving the success of the endodontic treatment.

Applications of laser has been widely spread in all fields of dentistrv. The diode laser has been established in dental practice because of its acceptable temperature rise as well as its bactericidal effect, and its relative economic price [7]. Moreover, it has been concerned recently in root canal treatment because of its bactericidal effect as well as its higher ability to penetrates the dentinal tubules to more than 1 mm of its thickness, surpassing the effect range of any other chemical disinfectant, that is thought to lead to better clinical outcomes [7].

Therefore, the aim of the present study was to evaluate the impact of contracted endodontic access cavity designs on root canal disinfection using diode laser.

The null hypothesis of this study was that there is no significant difference in the impact of contracted endodontic access cavities with diode laser disinfection versus conventional technique.

#### **Materials and Methods**

The following study was approved by the ethical review committee and institutional review board at Misr International University with IRB code: MIU-IRB-2223-192.

#### Study design

The current study was an in vitro study conducted on a total of 54 unidentified intact freshly human mandibular first molars extracted due to periodontal problems.

#### Sample size calculation

The total sample size was determined using power analysis for a Chi-square test for comparison between three groups and two subgroups. The effect size (w) was 0.75, using alpha ( $\alpha$ ) level of 0.05 (5%) and Beta ( $\beta$ ) level of 0.10 (10%) i.e. power = 90%; the minimum estimated sample size was a total of 54 subjects. Calculation was based upon the results of previous studies [2]. So, Total sample size is 54, so each group included 18 subjects.

#### Selection of samples

The teeth included in this study, were sound lower first molars, showing no cracks or caries with mature apices and moderate root curvature (10°-20°) according to Schneider method [8].

#### **Preparation of samples**

All teeth were decontaminated by immersion in 5.25% sodium hypochlorite for 30 min. Teeth were then cleaned and scaled to remove any surface deposits and/or calculus. Samples were mounted in standardized acrylic resin blocks using a mold with a dimension of  $1 \times 1 \times 2.5$ cm, then stored in normal saline solution at room temperature until the time of use.

#### Grouping of samples

Selected samples were divided into three equal groups (n=18) according to the access cavity design performed:

Group (1): Conventional Access Cavities

Group (2): Ninja Access Cavities

Group (3): Truss Access Cavities

Samples of each group were randomly sub-divided into two equal sub-groups (n=9) according to the disinfection method:

Subgroup (a): Diode laser disinfection was performed after Sodium Hypochlorite (2.5%) + EDTA 17% irrigation.

Subgroup (b): Sodium Hypochlorite (2.5%) + EDTA 17% was used for irrigation and disinfection.

Coded samples were used throughout the study to avoid possible bias.

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#### Access cavity preparation

Group 1: Conventional access cavity

The cavity was prepared by using a diamond round bur perpendicularly at the deepest point of the occlusal surface till reaching the pulp. Then complete de-roofing of the pulp chamber was performed exposing of all pulp horns and straight-line access into the canals was gained (Figure 1).



Figure 1. Conventional Access Cavity.

#### Group 2: Ninja Access Cavity

The initial access cavity was performed by using a diamond round bur perpendicularly at the deepest point of the occlusal surface, when the pulp chamber was reached, the cavity was slightly expanded bucco-lingually using a fissure bur. The mesio-distal length of the cavity was set to 2 mm; meanwhile, the bucco-lingual length of the cavity was 3 mm. This preparation usually starts at the central fossa of the occlusal surface, only as far as necessary to detect the canal orifices, preserving part of the pulp chamber roof [9] (Figure 2).



Figure 2. Ninja Access Cavity.

#### Group 3: Truss Access Cavity

The idea of this access cavity is to maintain part of the roof of the pulp chamber to achieve a more conservative opening. Two separate mesial and distal rounded cavities were prepared to approach the mesial and the distal canals. The access to the pulp chamber was gained from the occlusal surface to the roof of the pulp chamber by using a diamond round bur oriented parallel to the long axis of the tooth. The pulp chamber roof was intact between the mesial and distal access cavities [10] (Figure 3).



Figure 3. Truss Access Cavity.

#### **Bacterial Inoculation**

Before inoculation, samples were sterilized by autoclaving for 15 minutes at 121°C and were stored in physiological saline solution at 4°C until use.

Clinical isolate of E. faecalis from the Microbiology laboratory (Central laboratories, Ministry of Health, Eqvpt) was used for biofilm formation. The bacterial strain was inoculated in Brain Heart Infusion broth (BHI: Difco Laboratories, Detroit, MI, USA) and incubated at 37°C for 24 hours. The experimental suspensions were prepared by cultivating the biological marker on the surface of Brain Heart Infusion agar (BHIA; Difco Laboratories) following the same incubation conditions [17]. The bacterial cells were re-suspended in saline to reach a final concentration of about 3 x 10<sup>8</sup> cells/mL, adjusted to No. 1 MacFarland turbidity standard which was used to infect the samples.

The Enterococcus faecalis strain selected in this study was from the

American Type Culture Collection (ATCC 4083). E. faecalis strain was grown overnight at 37 °C in tryptic soy broth (TSB) supplemented with 1% glucose. Purity of culture was checked, and inoculum was adjusted in PBS to a turbidity of 0.5 McFarland scale (Figure 4).

Sterile pipettes were used to inoculate each specimen with 100  $\mu$ l of the bacterial suspension. Then the specimens were incubated at 37°C for 21 days.



Figure 4. SEM image of developed E. faecalis biofilm in the root canal

#### Root canal preparation and disinfection

In all groups, cleaning and shaping was performed using using Pro-Taper NEXT (Maillefer, Dentsply) to the full working length according to the manufacturer's instructions.

Sub-group (a): irrigation was performed throughout the procedure using NaOCI 2.5% followed by EDTA 17% (total 10 mL) between each file. Then diode laser (Epic X<sup>\*\*</sup>, BIOLASE, USA) was applied with a wavelength 940 nm, and an output power 1.5 Watt [7] (Figure 5). A 200  $\mu$ m non-initiated tip was used for root canal disinfection in a circular motion from apical to coronal (4 cycles each canal reaching the full working length).

Subgroup (b): irrigation was done using NaOCI 2.5% followed by EDTA 17% (total 10 mL) between each file.



Figure 5. Diode laser (Epic X<sup>™</sup>, BIOLASE, USA) with an output power 1.5 Watt

### **Bacterial Evaluation using Confocal** laser scanning microscope (CLSM)

All samples were cross sectioned horizontally at 2mm from the apex using 0.3 mm Isomet saw at 200 rpm and continuous water cooling. Then they were stained with BacLight stain Live/Dead (Invitrogen, Carlsbad, CA), and assessed for adherence of bacteria using confocal laser scanning microscopy set at the excitation/ emission wavelengths of 480/500 nm with the application of fluorescein diacetate dye, using a 40X magnification oil lens. Images were taken and used to quantify the live (green) and dead (red) bacteria using the Leica Application Suite-Advanced Fluorescence software (Figure 6-11). The measured red/green fluorescence intensities were used to calculate the percentage of dead bacteria over both dead and live bacteria [11].

#### Statistical analysis

All data was collected, tabulated and statistically analyzed. Numerical data were explored for normality by checking the data distribution and using Kolmogorov-Smirnov and Shapiro-Wilk tests. All data showed parametric (normal) distribution. Data were represented as mean, standard deviation (SD) and 95% Confidence interval for the mean (95% CI) values.

The Two-way Analysis of Variance (ANOVA) test was used to compare between percentage reductions in all groups. Tukey's post-hoc test was used for pair-wise comparisons when ANOVA test is significant.

The significance level was set at P ≤ 0.05. Statistical analysis was performed with IBM® SPSS® Statistics Version 20.

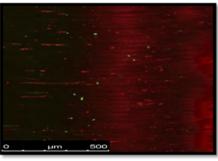


Figure 6: CLSM image of Conventional access with Diode laser.

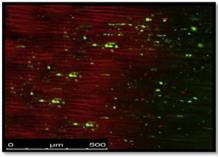


Figure 7: CLSM image of Conventional access with NaOCI.

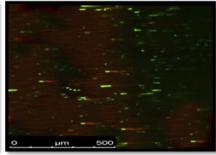
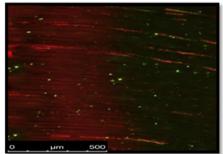


Figure 8. CLSM image of Ninja access with Figure 9. CLSM image of Ninja access with NaOCI. Diode Laser.



Diode Laser.

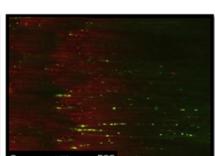


Figure 10. CLSM image of Truss access with Figure 11. CLSM image of Truss access with NaOCI.

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## Results

# Bacterial percentage reduction in Conventional Access Group:

Results have shown that there was no statistically significant difference in bacterial reduction with conventional access cavity, either using Diode laser or NaOCI irrigation (Table (1) & Figure 12).

# Bacterial percentage reduction in Ninja Access Cavity group:

Results showed that bacterial reduction in Ninja access cavity either using Diode laser or NaOCI irrigation showed also no statistically significant difference (Table 2 & Figure 13).

## Bacterial percentage reduction in truss access group:

There was no statistically significant difference in bacterial reduction in truss access cavity, either using Diode laser or NaOCI irrigation (Table 3 & Figure 14).

# Bacterial percentage reduction of using Diode laser disinfection:

Diode laser disinfection increased the bacterial reduction within each group, with comparable results among all groups. The highest bacterial reduction was seen in the conventional access design group, and the least bacterial reduction with the Ninja access cavity design group, but with no statistically significant difference between groups (Table 4 & Figure 15).

# Bacterial percentage reduction of using NaOCI irrigation:

The results have shown that the least statistically significant bacterial reduction was recorded in the Ninja access cavity design group in comparison to the conventional access cavity design group. However, this difference is statistically insignificant with the truss access design group (Table 5 & Figure 16).

## Bacterial percentage reduction of All groups:

Two-way ANOVA test showed that there was statistically significant difference between the groups (P-value <0.05) (Table 6 & Figure 17). Pair-wise comparisons between the groups using Tukey's test have shown that there is no statistically significant difference in bacterial reduction within each group either using Diode laser disinfection or Na-OCI irrigation.

Regardless of the access cavity design, using NaOCI irrigation have shown lower bacterial reduction in comparison to Diode laser disinfection with NaOCI irrigation, but with no statistically significant difference.

For access cavity designs, regardless of the type of disinfection used, conventional access cavities have shown the highest bacterial reduction, with the least bacterial reduction with Ninja access cavities.

#### Table 1: Descriptive statistics of Bacterial percentage reduction mean values in conventional access cavity group

|                                             |                  |                    |       | 95% CI      |             | P-value |
|---------------------------------------------|------------------|--------------------|-------|-------------|-------------|---------|
| Access Cavity Design Disinfection method Me |                  | Mean               | SD    | Lower bound | Upper bound |         |
|                                             | Diode laser      | 67.33 <sup>A</sup> | 8.737 | 45.63       | 89.04       |         |
| Ninja Access Cavity                         | NaOCI irrigation | 57.33 <sup>^</sup> | 5.859 | 42.78       | 71.89       | 0.761   |

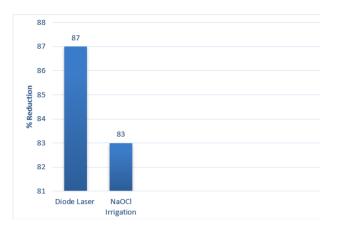
\*: Significant at  $P \le 0.05$ 

#### Table 2: Descriptive statistics of Bacterial percentage reduction mean values in Ninja access cavity group

|                      |                     | Mean    | SD     | 95%         |             |         |  |
|----------------------|---------------------|---------|--------|-------------|-------------|---------|--|
| Access Cavity Design | Disinfection method |         |        | Lower bound | Upper bound | P-value |  |
| Truss access cavity  | Diode laser         | 82.67 ^ | 14.189 | 47.42       | 117.91      | 0.885   |  |
| ,                    | NaOCI irrigation    | 74.67 ^ | 10.599 | 48.34       | 101.00      |         |  |

\*: Significant at  $P \le 0.05$ 

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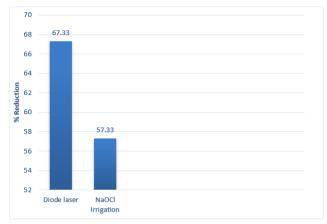


Figure 12. Bar chart representing mean and standard deviation values for bacterial percentage reduction in conventional access cavity group

Figure 13. Bar chart representing mean and standard deviation values for bacterial percentage reduction in Ninja access cavity group.

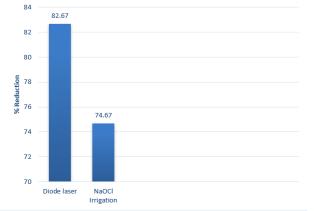
#### Table 3: Descriptive statistics of Bacterial percentage reduction mean values in truss access group.

| Access Cavity Design | Disinfection method | Mean               | an SD 95% Cl |             | СІ          | P-value |
|----------------------|---------------------|--------------------|--------------|-------------|-------------|---------|
|                      | Distinction method  | Ivicali            |              | Lower bound | Upper bound | -value  |
| Conventional Access  | Diode laser         | 87.00 <sup>A</sup> | 1.000        | 84.52       | 89.48       |         |
| Cavity               | NaOCI irrigation    | 83.00 <sup>A</sup> | 8.888        | 60.92       | 105.08      | 0.993   |

\*: Significant at  $P \le 0.05$ 

Table 4: Descriptive statistics and results of Two-way ANOVA and Tukey's tests comparison between Bacterial percentage reduction in the different groups (Diode laser disinfection) regardless of the access design used.

| Disinfection method Access Cavity D |                      | Maan               | CD.    | 95% CI      |             |         |
|-------------------------------------|----------------------|--------------------|--------|-------------|-------------|---------|
| Distinection method                 | Access Cavity Design | Mean               | SD     | Lower bound | Upper bound | P-value |
|                                     | Conventional         | 87.00 <sup>A</sup> | 1.000  | 84.52       | 89.48       |         |
| Diode laser                         | Ninja                | 67.33 <sup>A</sup> | 8.737  | 45.63       | 89.04       | 0.684   |
|                                     | Truss                | 82.67 ^            | 14.189 | 47.42       | 117.91      |         |





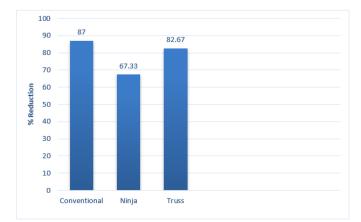


Figure (15): Bar chart representing mean and standard deviation values for bacterial percentage reduction in Diode laser disinfection

Table 5: Descriptive statistics and results of Two-way ANOVA and Tukey's tests comparison between Bacterial percentage reduction in the different groups (NaOCI irrigant) regardless of the access design used.

| Disinfortion worth od | Assess Covity Design | Mean               | SD     | 95% CI      |             |         |
|-----------------------|----------------------|--------------------|--------|-------------|-------------|---------|
| Disinfection method   | Access Cavity Design | wean               |        | Lower bound | Upper bound | P-value |
|                       | Conventional         | 83.00 <sup>A</sup> | 8.888  | 60.92       | 105.08      |         |
| NaOCI                 | Ninja                | 57.33 <sup>в</sup> | 5.859  | 42.78       | 71.89       | 0.035*  |
|                       | Truss                | 74.67 AB           | 10.599 | 48.34       | 101.00      | 1       |

\*: Significant at P ≤ 0.05, Different superscripts in the same column indicate statistically significant differences according to Tukey's test

#### Table 6: Descriptive statistics and results of Two-way ANOVA and Tukey's tests comparison between Bacterial percentage reduction in the different groups

| Disinfection method  | Diode Laser        |        | NaOCI i             | P-value |        |
|----------------------|--------------------|--------|---------------------|---------|--------|
| Access Cavity Design | Mean               | SD     | Mean                | SD      |        |
| Conventional         | 87.00 <sup>A</sup> | 1.000  | 83.00 <sup>A</sup>  | 8.888   |        |
| Ninja                | 67.33 AB           | 8.737  | 57.33 <sup>в</sup>  | 5.859   | 0.015* |
| Truss                | 82.67 ^            | 14.189 | 74.67 <sup>AB</sup> | 10.599  |        |
| P-value              | 0.684              |        | 0.035*              |         |        |

\*: Significant at P ≤ 0.05, Different superscripts in the same column indicate statistically significant differences according to Tukey's test

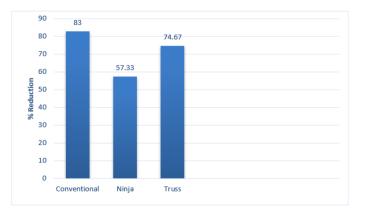


Figure (16): Bar chart representing mean and standard deviation values for bacterial percentage reduction in NaOCI irrigation

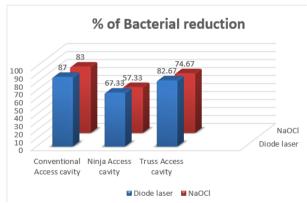


Figure (17): Bar Chart representing mean and standard deviation values for bacterial percentage reduction in all groups

### Discussion

A successful endodontic treatment depends on accurate access cavity preparation to the pulp chamber and the root canal system [12]. Since an appropriate access cavity leads to efficient procedures such as canal detection, localization, chemo-mechanical preparation, and obturation [13]. The conventional endodontic access cavity design includes all pulp horns and deroofing of the pulp chamber so that the coronal portion of the root canal system is sufficiently removed [14].

These endodontic access cavity designs and unnecessary loss of tooth structure could greatly affect the fracture resistance of endodontically treated teeth. The remaining dental structure is controlled by the endodontic access cavity preparation. So, new techniques to conventional access cavities, as minimally invasive access cavities have been introduced [15].

The main objective of minimal access cavities is to preserve as much tooth structure as possible and thus increasing the fracture resistance of endodontically treated teeth [16, 17]. Ninja endodontic access cavities and truss access cavities are two different designs of the minimally invasive access cavities. Ninja endodontic access involves minimal removal of tooth structure, partially preserving the roof of the pulp chamber. The truss access cavity is two separate cavities in multirooted teeth for maximum preservation of dentin between the cavities [14, 16].

In ideal form, irrigants should have antimicrobial action, tissue-dissolution activity, demineralization, lubrication, and ability to remove smear layer and debris (Zhender et al, 2006). As NaOCI has an excellent antimicrobial action, and tissue solubility, it has been used in endodontics as the most common irrigation solution. One important limitation of NaOCI is its inability for smear layer removal affecting its antibacterial efficiency and its penetration deeper in the dentinal tubules [17].

Various adjunctive approaches have been suggested to improve disinfection during root canal treatment procedures. Some of the disadvantages can be partly solved using ultrasound activation or photoactivation systems that enhance penetration and lead at least to some improvement in the antimicrobial activity of rinsing solutions [18]. Laser-based methods have also been developed in recent years and have been reported to be effective for root canal disinfection [19]. The Nd:YAG laser, with a wavelength of 1064 nm, was one of the first lasers used for root canal disinfection [20].

Nowadays, diode lasers with wavelength ranges of around 940– 980 nm are mainly used for the purpose, and studies have shown adequate bacterial reduction with these [21]. Moreover, diode laser was chosen as a disinfectant system because laser achieves better disinfection of root canal system by deep penetrating and cleaning complex endodontic system [22].

Results of the current study showed bacterial reduction in all groups either using Diode laser or NaOCI irrigation, with no significant difference. Since irrigation and disinfecting of the root canal is the key element in elimination of bacteria. Studies have showed that copious irrigation with an antimicrobial solution during mechanical root canal preparation has an essential effect on the reduction of intraradicular microorganisms [23]. Sodium hypochlorite (NaOCI) is the most widely used irrigant solution and it has excellent antimicrobial action and tissue solubility. Also using Diode laser in root canal disinfection is claimed to achieve better disinfection of root canal system by deep penetrating and cleaning complex endodontic system [19, 23].

This was in accordance with previous studies that reported that efficient irrigation plays crucial role in increasing bacterial reduction [24-26]. This reinforces the thought that using thorough irrigation will lead to effective disinfection of the root canal system, making the role of the access cavity limited. This could be also attributed to the instrumentation of the root canals, that contributes to increasing microbial reduction, as it provides a room for efficient irrigation in the canal [6, 27].

Regardless of access cavity design, the results of bacterial percentage reduction using different disinfection methods, have shown that Diode laser disinfection combined with NaOCI irrigation increased the bacterial reduction within each group. This could be contributed to the antibacterial effect of laser irradiation, which is based on the thermal properties of the laser-tissue interaction [20]. Due to the favorable absorption spectrum of Diode laser, it can penetrate deeply into the surrounding root dentin. Due to the high level of absorption into the color components of the bacteria, the laser energy is selectively absorbed and released locally as heat, causing the bacteria themselves to be killed by the increase in temperature. An antibacterial effect can thus also be achieved even in deep tissue layers and at the base of the dentinal tubules [20, 21]. In addition, recently published studies, stated that penetration depths of 1000  $\mu$ m into the depth of the surrounding root canal dentin are reached by the laser light, which is significantly greater than the penetration depth with conventional rinsing solutions<sup>[23]</sup>.

Although, the use of rinsing solutions such as sodium hypochlorite (NaOCI) is regarded as the gold standard for disinfection in endodontic treatments due to their good antibacterial efficacy and ability to remove the smear layer. However, conventional rinsing can be affected by anatomical features and mechanical problems in the conventional rinsing process [28]. Another major limitation of the disinfection effect with conventional rinsing solutions, which has been widely discussed in the literature, is their limited depth of penetration into the dentin surrounding the root canal. Studies have demonstrated that microorganisms can invade the periluminal dentin up to a depth of 1100  $\mu$ m [29]. However, penetration depths of no more than 160  $\mu$ m into the dentin have been reported for chemical irrigants used during endodontic treatment procedures [28]. Such irrigants are therefore unable to eliminate bacteria that have penetrated the deeper dentin layers [23], and this may lead to recurrent endodontic lesions.

In addition, the results of the current study revealed that Ninja access cavities with NaOCI irrigation showed the statistically significantly lowest mean bacterial percentage reduction. While conventional access cavity either using Diode laser or NaOCI irrigation showed the highest bacterial reduction with mean values 87 and 83 respectively, followed by Truss access cavity with Diode laser disinfection, and NaOCI irrigation with no significant difference. This might be justified by the fact that conservative access cavity creates considerable coronal interferences, which would hinder proper instrumentation and cleaning of the root canals. Unlike Ninja access cavity, and despite being a minimally invasive technique, the truss access cavity offers a more free and direct access to the root canals, which may explain the better results obtained, recording mean values of 82.67 for Diode laser disinfection and 74.67 for NaOCI irrigant. This reinforces the concept that minimally invasive endodontic accesses might jeopardize the overall success in reducing the microbial load during root canal preparation, especially when compared to the conventional access cavities [30-32].

Conventional access cavity and truss access cavity groups had statistically comparable results, possibly due to the fact the truss access cavity design provides a more direct access to mesial and distal canals, having two separate cavities, when compared to the Ninja access, which creates considerably more coronal interferences during instrumentation [31]. These coronal interferences could be responsible for insufficient elimination of bacterial count that occurred in specimens of the Ninja Access Cavity group, especially with NaOCI irrigant, since the mechanical instrumentation is affected by the constricted access cavity design which would adversely affect the cleaning ability of the irrigant [14].

Another recent research was performed to evaluate using different endodontic access cavity designs on the elimination of Enterococcus faecalis from the root canal systems. The results were also in accordance with our study, concluding that these new modalities of access cavity designs jeopardize the cleaning ability and instrumentation of the root canal system [33:35].

It is imperative to highlight that this study assessed the impact of contracted endodontic access cavities with diode laser disinfection in infected root canals. This is a crucial factor in determining the overall success of root canal treatment and may affect the choice of a given endodontic access cavity and the type of disinfection used. The goal of root canal treatment is the disinfection of the root canal system and here, once more, no evidence of any advantages to support the use of minimally invasive access cavities has been found, especially with the usage of conventional irrigation techniques [36].

Although combining Diode laser disinfection with NaOCI irrigation could yield to better outcomes, and despite the claims of the potential benefits these techniques could bring, yet still further studies and more evidence are recommended to support their use and consequently to justify the recommendation for practitioners to use these new techniques in their everyday clinical procedures.

#### Conclusions

Within the limitations of the current study, the following can be concluded:

- Contracted endodontic access cavities did not offer any advantages in comparison with the conventional endodontic access cavities regarding microbial reduction and cleaning ability.
- 2. Diode laser disinfection with Sodium hypochlorite irrigation is effective in microbial reduction regardless of endodontic access cavity design used.

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