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The effect of different access cavity designs on bacterial biofilm reduction using Er,Cr:YSGG laser in comparison to conventional disinfection protocols: An In-Vitro study

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ABSTRACT

Background: There is minimal scientific evidence concerning the effect of contracted endodontic access cavity design (CEC) combined with Erbium, Chromium: yttrium-scandium-gallium garnet (Er,Cr:YSGG) laser on intracanal bacterial biofilm reduction. Traditional endodontic access cavity preparation (TEC) provides straight-line access to the canal orifices. However, more dentin tooth structure is removed, which may affect resistance to tooth fracture under cyclic loading. ***Aim:*** To evaluate the effect of access cavity design on the reduction of bacterial biofilm using different disinfection protocols. ***Materials and Methods:*** Sound extracted non-restored maxillary first permanent premolars were selected. The samples were divided into two main equal groups according to access cavity design. Each group was subdivided into 3 equal subgroups according to root canal disinfection protocol: subgroup A: saline, subgroup B: 5.25% NaOCl,17%EDTA, Subgroup C: Er,Cr: YSSG Laser. Samples were then examined after the final disinfection protocol for biofilm bacterial reduction using microbiological culture. ***Results:*** Traditional access showed significantly higher bacterial reduction than contracted access regarding saline and laser disinfection, while there was no statistically significant difference between both cavity designs using NaOCl. ***Conclusion****:* Access cavity design exhibited direct influence on bacterial reduction using Er,Cr: YSGG laser. However, access cavity designs had no influence on bacterial disinfection using NaOCl.

Keywords: Laser, access cavity, contracted access, bacterial disinfection, Erbium.

INTRODUCTION

The cleaning and shaping process is considered a very important and critical step in endodontic treatment. Still it must be proceeded by proper coronal cavity preparation to achieve adequate and effective access to root canal space. Proper access permits the detection of all root canal orifices and allows the instrumentation and irrigation techniques to remove organic pulpal tissue and infected inorganic dentine from the pulp spaces.[1,2]

Contracted endodontic access cavity designs (CEC), in addition to ultraconservative designs as ninja access and truss access, have been proposed in the past few years in order

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to preserve part of the chamber roof and cervical dentine for higher fracture resistance, thus increasing long-term survival and function of endodontically treated teeth.[3,4]

Controversy exists concerning the effect of recent contracted access cavity designs on bacterial disinfection in comparison to traditional access cavity.

Sodium hypochlorite solution is, to date, the gold standard root canal irrigant. NaOCl is the only available irrigant that provides organic tissue dissolvent action with broad-spectrum antimicrobial activity. It also acts as an efficient lubricant for mechanical instrumentation and can flush loose debris from root canal space.[5]

Laser technology has been proposed as an effective auxiliary tool in disinfection of root canal space by having the ability to diffuse and penetrate inside dentinal tubules more than 1000 um.[6] The Erbium, Chromium: yttrium-scandium-gallium garnet (Er,Cr:YSGG) has a wavelength of 2780 nm, which is delivered through radial firing tips at different output powers. The high affinity of this type of Laser to hydroxyapatite and water produces cleaner root canal walls when compared to other types.[7] Many studies tested the efficacy of (Er,Cr:YSGG) Laser disinfection against root canal microorganisms and reported that its use alone reduced the number of viable bacteria such as *E.faecalis* inside root canal space.[8] However, there is no reported study up to the date of publication that combined the use of Er,Cr YSGG laser along with contracted access cavity designs.

So, the aim of this study is to compare traditional endodontic access cavity design (TEC) and contracted endodontic access cavity design (CEC) using NaOCl irrigation and (Er,Cr: YSGG) Laser irradiation, testing their influence on the reduction of intracanal bacterial biofilm.

MATERIALS AND METHODS

1.Study setting:

This study was approved by the Research Ethical Committee (REC) of the Faculty of Oral and Dental Medicine, Misr International University, with approval **MIU-IRB-2122-131**. It was carried out at MIU Microbiology laboratory and MIU Dental Laser Center on a total of 60 unidentified freshly extracted intact human maxillary first premolar teeth from MIU teeth bank. Patients from whom these teeth were extracted signed a consent form for the future use of their teeth in scientific research after extraction.

2.Sample size calculation:

This power analysis used a percentage reduction in bacterial counts as the primary outcome. Power analysis was conducted for a 2 x 3 fixed effects analysis of variance. The

first factor (Access cavity design) includes two levels, and the second factor (Irrigation protocol) includes three levels. Based upon the results of Tufenkci P and Yilmaz K (2019) and Eldeniz AU et al. (2007), the effect size (f) for the first factor was (1.45) and for the second factor (f) was (46.17). Using an alpha (α) level of (5%) and Beta (β) level of (20%) i.e.power = (80%), the minimum estimated sample size was (7) samples per cell, giving a total of (42) samples. Sample size calculation was performed using IBM® SPSS® SamplePower® Release 3.0.1.

3. Sample selection:

Inclusion criteria:

Sound, non-restored, extracted maxillary permanent first premolars with closed apices. The root canal curvature was measured using Schneider's method. Only those roots with an angle of curvature ranging from 10 to 20 degrees were selected.[9]

Exclusion criteria:

Teeth with restorations, caries, fracture, calcifications, internal or external resorption, open apices, and teeth with more than two canals were excluded.

4. Samples Grouping:

A total of sixty samples were selected, and coded samples were used throughout the study to avoid possible bias. Samples were classified into two main equal groups (n=30) according to access cavity design:

Group 1- Traditional access cavity

Group 2- Contracted access cavity

Then, each group was subdivided into three equal subgroups (n=10) according to the final root canal disinfection protocol (**Figure 1)**

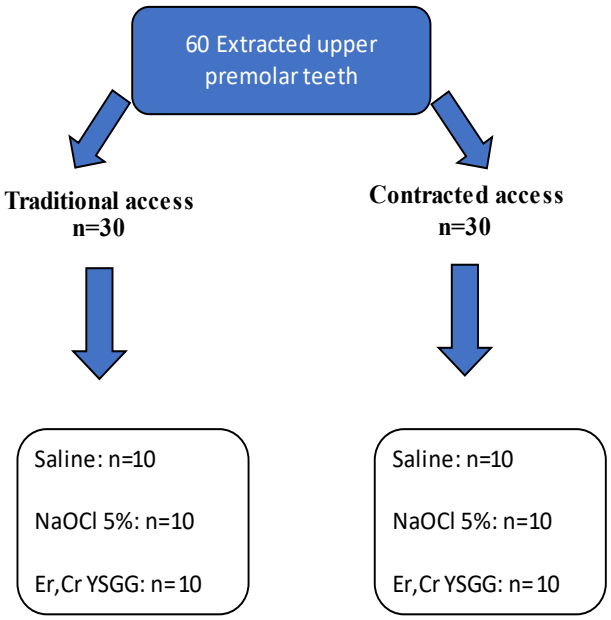

Figure (1): diagram showing samples grouping.

- Subgroup A: Saline (n=10)
- Subgroup B: 5.25% NaOCl, 17% EDTA (n=10)
- Subgroup C: Er,Cr:YSSG Laser (n=10)

5. Samples Preparation:

- Soft tissue tags and calculus were removed using a periodontal curette. Teeth were then washed under running water and stored in saline solution until use.

6. Endodontic Access cavity preparation:

- Traditional access cavity Design: Half of the samples (thirty teeth) was accessed according to traditional guidelines using a diamond round bur perpendicularly at the deepest point of the occlusal surface. After reaching the dentin, the pulp was accessed using a #4 steel round burs. Complete deroofing of the pulp chamber with exposure of all pulp horns and divergent cavity walls was done using round end diamond stone size #3. (**Figure 2)**

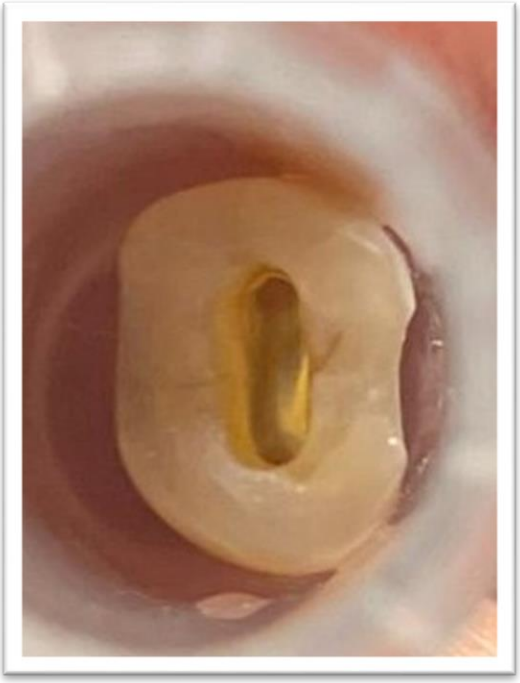

Figure (2): photograph showing traditional access cavity design.

- Contracted access cavity Design: The other half of the samples (thirty teeth) was accessed conservatively under a dental operating microscope to reach the orifices of the canals while preserving part of the pulpal roof and per cervical dentin as much as possible with convergent cavity walls using round burs and diamond round end stone size #2.[10,11] (**Figure 3)**

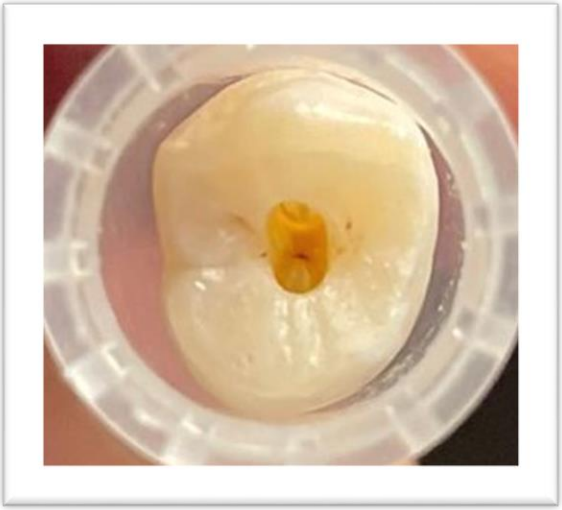

Figure (3): photograph showing contracted access cavity design.

7. Initial root canal preparation:

- Each root canal was negotiated using a flex-o-file ISO #10 to achieve patency.
- Using an ISO #15 file, the working length was determined, where the file was introduced into the canal until it was seen flushing with the apical foramen. The file was measured, then 1 mm was subtracted and recorded as working length, then root canals were irrigated by 5.25%NAOCL.(2,11)
- Root canals were flared coronally using the orifice opener file of the Protaper gold rotary system (PTG) according to the manufacturer's instructions.

- The effect of NaOCl was neutralized using a sterile saline solution, and all samples were wrapped in moisture gauzes with saline and placed in sterilization pouches.
- Pouches were placed in the autoclave for 20 minutes at 120°C.

8. Biofilm preparation:

- Preparation of 24 hours culture of the tested bacterial reference strain *E.faecalis* in 10 mL of Brain Heart Infusion broth (BHI) or Tryptone Soy Broth (TSB) and incubated at 37°C for 24 hours.
- The Turbidity was adjusted to No. 1 MacFarland turbidity with physiological saline or broth.
- The turbidity of the environment confirmed the growth of *E.faecalis* bacteria during the incubation period. Apices were sealed using nail polish to prevent leakage of bacteria and create a closed-end system simulating the vapor lock effect.[2,11]
- One ml of tested bacterial suspension was inoculated in the root canals then incubated at 37°C for 72 hours.
- The intracanal solution was removed with a suitable syringe.
- A 24hr pure culture suspension (1 McFarland) of the tested strain *E.faecalis* was introduced inside the prepared canal; the process was repeated every 72 hours for 21 days.[12]
- Bacterial count at baseline for each premolar tooth was counted and recorded before further root canal manipulation.

9. Root canal shaping:

All mechanical instrumentation for all teeth in the two main groups was performed inside laminar air flow using the same rotary system (PTG) according to the manufacturer's instructions up to finishing file (F2) with an apical size of #25 and 0.08 variable taper. Sterile saline irrigation was used in between each instrument exchange throughout mechanical preparation (**Figure 4).**

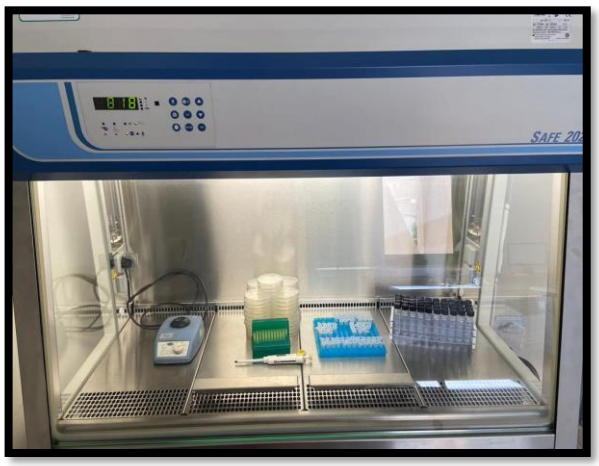

Figure (4): photograph showing laminar air flow.

10.Post instrumentation first bacterial count (S1):

The first bacterial count was recorded after finishing the mechanical instrumentation

by introducing sterile paper points into the root canals and maintaining it for 1 min for sample collection, using two paper points to collect from each sample, then transferred in 1 ml saline. Colony count for each paper point was performed on an agar medium.[11]

11. Root canal final disinfection protocol:

A) Groups 1A and 2A (Control groups):

Five mL of saline solution was introduced inside the root canals using a 30-gauge endodontic side vented needle (Navi tip) and kept for 5 minutes.

B) Groups 1B and 2B:

Five mL of 5.25% NaOCl sol followed by 5 mL of 17% EDTA solution using a side vented needle (Navi Tip) inserted 1 mm short from the working length and kept for 5 minutes.

C) Groups 1C and 2C:

(Er,Cr:YSSG) Waterlase Laser was used for the final canal disinfection protocol using the following settings (2780 nm, pulse duration of 60µsec, at power 1.5wtt, water 40%, air 20%). Laser activation was done using a gold handpiece and radial firing tip 2 (200 microns) (RFT) after the dryness of root canals using paper points. The radial firing tip was introduced inside the root canal, reaching 2 mm shorter than the working length, then used for 1-second intervals for each 2 mm for a total of 4 cycles for each root canal (**Figures 5, 6).**

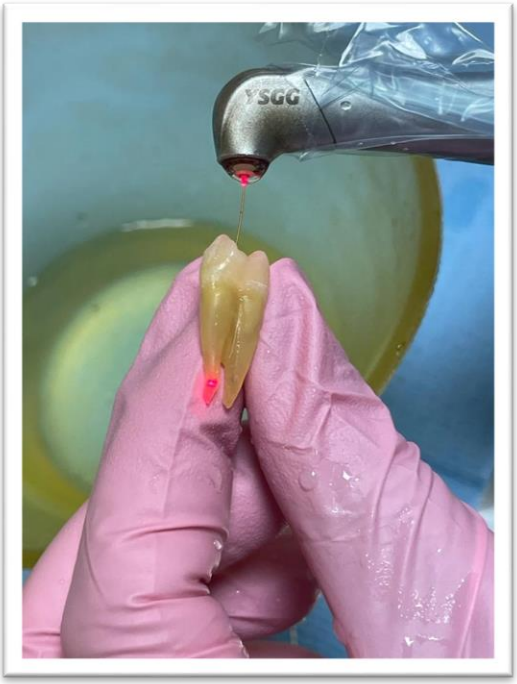

Figure (5): Photograph showing laser irradiation using RFT 2.

Figure (6): Photograph showing Water Laser device.

12. Post disinfection protocol second bacterial count (S2):

Sterile paper points were used to take bacterial samples from root canals and then transferred into tubes containing 0.85% sterile saline solution. Paper points were transferred into tubes with 1 mL 0.85% phosphate buffered solution vortexed for 1 minute. After 10 times serial dilutions within the sterile saline solution, 0.1-mL samples were cultivated on Columbia Agar sheep blood. The cultivated samples were incubated at 37°C for 24 hours, and the number of bacterial colonies was counted as (CFUs/mL) after root canal disinfection protocol.

Statistical analysis:

Numerical data were presented as mean and standard deviation (SD) values. They were explored for normality by checking the data distribution, and by using Shapiro-Wilk's test. Data were normally distributed and were analyzed using one-way ANOVA followed by Tukey's post hoc test. The significance level was set at $p<0.05$. Statistical analysis was performed with R statistical analysis software version 4.3.1 for Windows.[1]

RESULTS

Effect of access cavity design on the bacterial reduction:

The effect of access cavity design, along with each disinfection protocol on bacterial biofilm reduction percentage, is presented in **Table (1) and Figure (7)**.

Table (1): Effect of access cavity on bacterial reduction with different disinfection protocols.

Disinfection protocol	Percentage change of bacterial count (%) (mean±SD)		
	Group 1 Traditional Access	Group 2 Contracted Access	p-value
Subgroup (A) Saline	47.03±2.76	26.25±2.78	**<0.001***
Subgroup (B) NaOCl	100.00±0.00	100.00±0.00	**NS**
Subgroup (C) Laser	66.67±11.74	52.03±1.59	**0.001***

**; significant (p ≤ 0.05)*
ns; non-significant (p>0.05)

The amount of bacterial reduction in group 1 (traditional access) was significantly higher (47.03±2.76) than the bacterial reduction in group 2 (contracted access)

[1]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/.

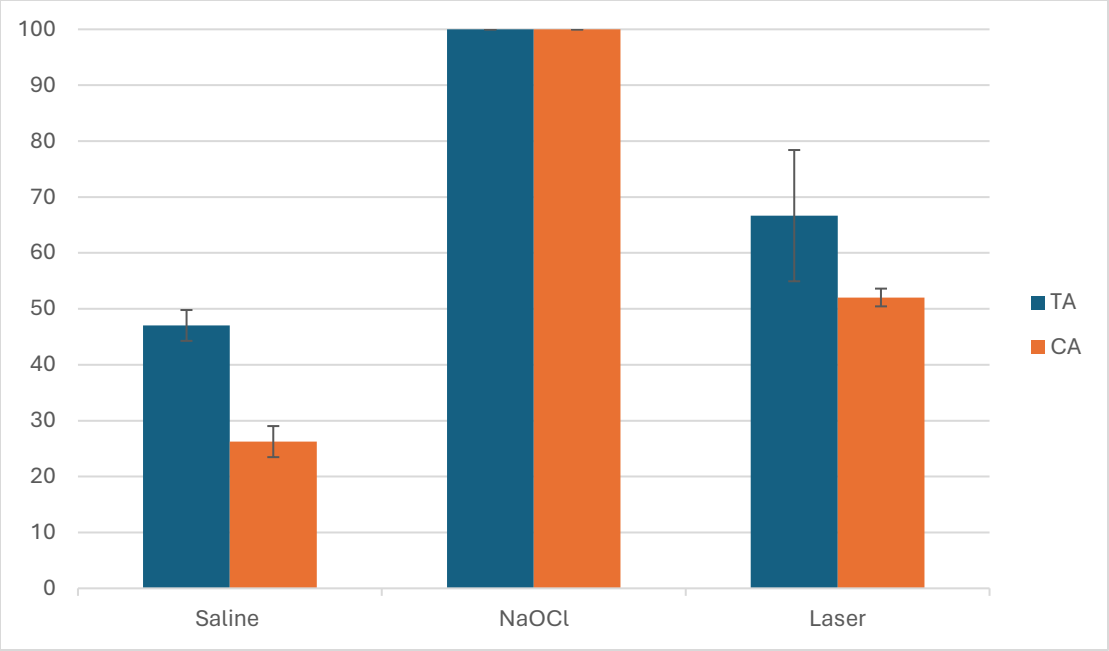

Figure (7): Bar chart showing mean and standard deviation values of percentage change of bacterial reduction in traditional (TA) and contracted access cavity (CA) designs after cleaning and shaping using saline, NaOCl and laser.

(26.25±2.78) when saline was used as the final root canal disinfection protocol (subgroup A).

Samples of (subgroup B) where NaOCl was the final canal disinfection protocol, showed 100% bacterial reduction in both tested groups. Similar to subgroup A, samples of (subgroup C) in which laser was the final canal disinfection protocol demonstrated significant bacterial reduction (66.67±11.74) with traditional access in comparison to contracted access (52.03±1.59). **(Figures 8, 9, 10, 11 & 12)**

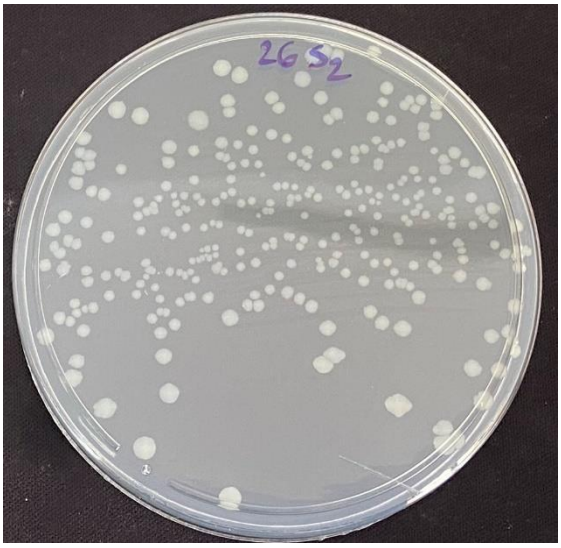

Figure (8): Agar plate showing bacterial count after saline irrigation in Group 1.

DISCUSSION

The aim of the present study was to address the limited evidence on the influence of conservative endodontic access cavity design on root canal disinfection. The choice of maxillary first premolar teeth is based on their susceptibility to mechanical failure and

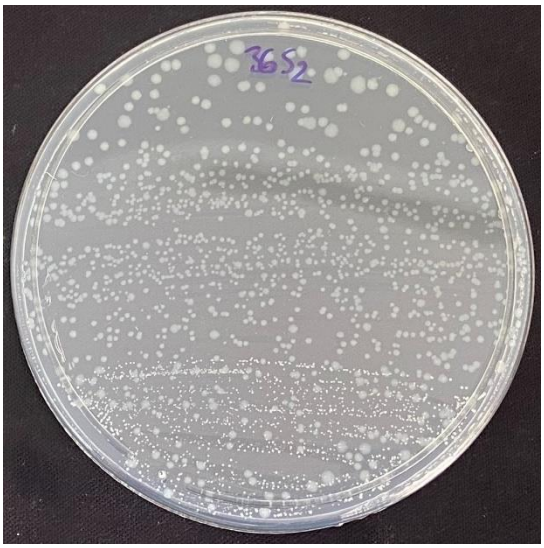

Figure (9): Agar plate showing bacterial count after saline irrigation in Group 2.

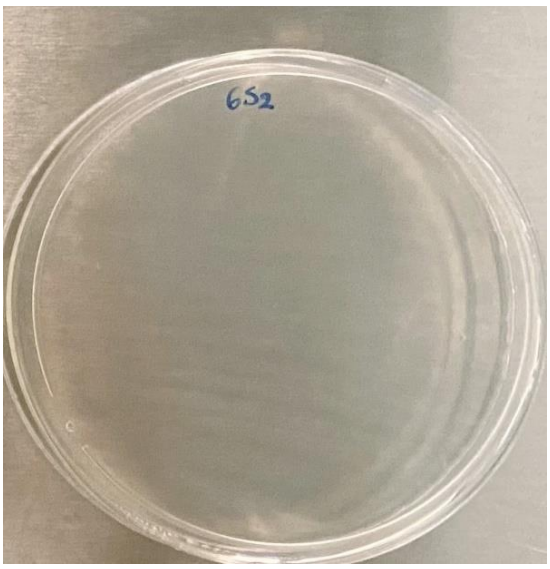

Figure (10): Agar plate showing complete bacterial eradication after 5.25% NaOCl irrigation.

fracture post-root canal treatment. The contracted access design represents a moderate approach between ultraconservative and traditional designs.[13,14]

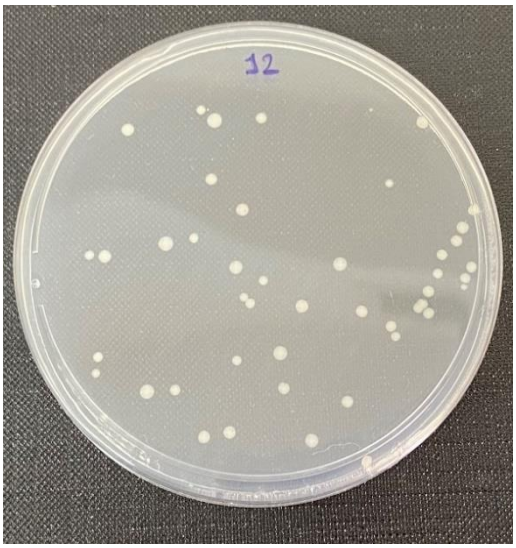

Figure (11): Agar plate showing bacterial count after laser disinfection in Group 1.

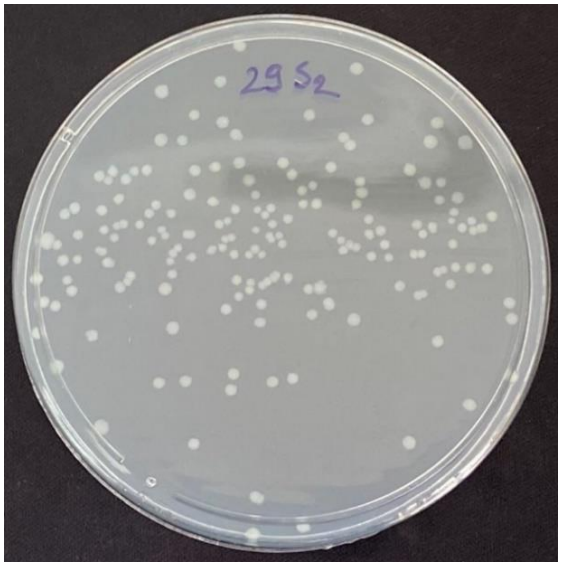

Figure (12): Agar plate showing bacterial count after laser disinfection in Group 2.

E.faecalis microorganism was selected in the present study for its frequent persistence in root canal infections. Bacterial culturing inside root canals for 21 days was employed to ensure the formation of well-

established bacterial biofilm mimicking clinical conditions.[15,16]

The study included the use of sodium hypochlorite (NaOCl) as the gold standard irrigant and evaluated the effectiveness of (Er,Cr:YSGG) laser for root canal disinfection. The Er,Cr:YSGG laser's mechanism involves rapidly growing and collapsing water vapor bubbles, producing high-velocity water jets along canal walls.[17,18]

Closure of the apex of teeth with transparent nail polish was done to simulate the in-vivo condition where the root is enclosed within a bony socket, resulting in gas entrapment in the apical third, producing what is known as the vapor lock phenomenon.[2]

The Colony Forming Unit (CFU) test was used to assess microbial reduction. The use of CFU allowed for multiple and sequential testing of the same sample at different interventional points and, hence, was more suitable for the applied study design.[11,19,20]

Regarding access cavity design and mechanical instrumentation with saline, traditional access samples exhibit higher bacterial reduction than contracted access samples. This is attributed to coronal restriction in contracted access, interfering with efficient mechanical instrumentation.

This was in accordance with **Andac et al**.[20] who concluded that the least bacterial reduction in contracted access in comparison to traditional access design was due to the absence of straight- line access, which affected mechanical instrumentation.

This was opposite to the study by **Tufenkci and Yilmaz,**[11] who revealed no significant difference between contracted and traditional access cavities on the elimination of *E.faecalis* following mechanical instrumentation, which might be due to different root anatomy where they utilized lower first molar teeth and different rotary systems.

Regarding the effect of the design of the access cavity on root canal disinfection when using Er,Cr YSGG laser, the traditional access cavity samples had significantly higher bacterial reduction than contracted access cavity samples. Although the laser radial firing tip diameter was smaller than 30 gauge needle, ensuring the full penetration to 2 mm shorter than the working length in the contracted access group, there were significantly higher bacterial residues in the contracted access group which might have been attributed to pulp tissue remnants inside the pulp chamber and root canal harboring *E.feaclais.* Consequently, the shortcoming of the laser action can be explained by the fact that the erbium laser was not able to dissolve organic pulp tissue but only removed the smear layer and eradicated microorganisms through

cavitation and thermal effects. As previous studies revealed, the design of the access cavity may compromise the instrumentation of the canals, leaving a higher percentage of untreated canal areas and pulpal tissue remnants which could potentially affect thorough disinfection and impede effective bacterial elimination.[21,22]

Only two studies utilized laser irradiation along with contracted access cavity design. The results were in accordance with the study by **Eltayeb and Nabeel**[23] that used a diode laser in root canal disinfection and revealed that minimal invasive designs restricted the access cavity with coronal interference, which limited the flow of irrigants apically and impaired the elimination of microorganisms in the root canal system. However, the results of the latter were in contrast with **Shan et al.**[19] study in which minimal and traditional access cavity designs showed significant difference only while using conventional irrigation alone but both designs showed comparable results and bacterial reduction when NaOCl irrigant was activated using PUI and LAI using Er:YAG laser.

Regarding the effect of the design of the access cavity on root canal disinfection while using 5.25% NAOCL and EDTA 17%. Both cavity designs showed complete bacterial eradication of *E.faeclais* with no significant difference between the groups. This could be attributed to the known dissolving and antimicrobial action of NaOCl against even the most resistant species. In addition to the combination of NaOCl and EDTA, which is the best for smear layer removal, it is known to be heavily loaded with microorganisms.

The results were in accordance with many previous studies[24–26] in which they all showed complete bacterial disinfection while using a full concentration of 5.25% NaOCl, but none of them included the effect of access type as a comparable factor. The results, on the other hand, were in contrast with **Vieira et al.,**[2] who studied the effect of access cavity design on different teeth anatomy utilizing lower incisor teeth using lower concentration of 2.5% of NaOCl and, **Shan et al**.[19] study in which minimal and traditional access cavity designs showed significant difference while using conventional irrigation but again with lower concentration 1% of NaOCl on upper maxillary molar teeth. **Girgis et al.,**[27] used a different method of evaluation of bacterial disinfection through Brown and Brenn staining method in which a light transmission microscope was used in order to detect the bacterial colonies inside dentinal tubules.

In summary, the research contributes insights into the efficacy of different access designs, irrigants, and laser techniques for root

canal disinfection, emphasizing the importance of proper instrumentation and disinfection protocols in endodontic treatment.

CONCLUSIONS

Based on the results of the present study, it could be concluded that NaOCl irrigation is still the most effective root canal disinfection method, irrespective of access cavity design. The use of erbium laser as a disinfection tool in cases of contracted access cavity designs did not prove to be efficient enough to be used alone.

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CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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