ORIGINAL ARTICLE

Evaluation of Antimicrobial Efficacy and Penetration Depth of Various Irrigants into the Dentinal Tubules with and without Lasers: A Stereomicroscopic Study

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Abstract

Aim: To evaluate and compare the antibacterial efficacy and horizontal depth of penetration of various irrigants into the dentinal tubules when used alone and when combined with lasers.

Materials and methods: An experimental study was done on 42 single-rooted teeth. Access cavity preparation was done and the canals were enlarged up to a ProTaper file size F2 of length 25 mm. They were inoculated with 0.1 mL of *Enterococcus faecalis* and the samples were randomly assigned into six different groups of seven teeth each. The following irrigation systems were used individually and in combinations—normal saline, sodium hypochlorite, chlorhexidine gluconate, diode laser and erbium, chromium:ytrrium scandium gallium garnet laser (Er,Cr:YSGG laser). The colony-forming units (CFU) of bacteria before and after disinfection and the penetration depth of different groups were determined. Statistical analysis was done by an ANOVA test.

Results: The highest number of CFU of bacteria was shown by the group where saline was used and it also showed the least penetration depth compared to that of the Er,Cr:YSGG laser group.

Conclusion: Er,Cr:YSGG laser when used along with sodium hypochlorite and chlorhexidine gluconate showed the highest reduction in the CFU of bacteria and the greatest penetration depth when observed under a stereomicroscope.

Clinical significance: Laser-assisted irrigation regimes have a high antibacterial efficacy and more penetration depth into the dentinal tubules.

Keywords: Diode laser, Erbium, Chromium:yttrium scandium gallium garnet laser, Experimental study, Penetration depth.

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Introduction

One of the most neglected phases of endodontic therapy is the eradication of microorganisms and the complete removal of organic debris, necrotic pulp tissue, pulpal remnants, and dentinal shavings from the root canal system. Microorganisms proliferate to form microcolonies; the necrotic pulp provides a space for microbial colonization, giving them a moist, warm, nutritious, and an anaerobic environment. Even with numerous advances in rotary endodontics, failures in root canal treatment exist. The success of endodontic therapy depends upon the ability to remove these microorganisms and prevent flare-ups.

In order to increase the efficacy of mechanical preparation, instrumentation should always be supplemented with active irrigating solutions. The objectives of irrigation are both mechanical and biological. The mechanical objectives include flushing out of debris, canal lubrication, and dissolving both organic and inorganic tissues. The biologic function of the irrigants is related to their antimicrobial effect.[1] An ideal endodontic irrigant should have antimicrobial action, should mechanically flush out debris, should be non-toxic and biocompatible, and should dissolve the necrotic and vital pulp tissues. It should be able to serve as a lubricant, remove the smear layer, and must have a low surface tension.[2]

A report by Sundqvist et al. concluded that *Enterococcus faecalis* (a Gram-positive facultative anaerobe) is the most commonly recovered species from endodontic cases requiring retreatment. It has the ability to survive as a single organism without the support of other bacteria[3] and can infect dentinal tubules up to 800 µm from the root canal. It is resistant to calcium hydroxide treatment. It has been postulated that a virulence factor of this microorganism may be related to its ability to invade dentinal tubules and adhere to collagen. Thus, recognizing the potential role of *E. faecalis* in the failure of endodontic therapy makes it important to develop strategies to control infections caused by this microorganism.

Sodium hypochlorite is the most commonly used irrigating solution and is an excellent antibacterial agent capable of dissolving necrotic tissue, vital pulp tissue, and organic components of dentin and biofilms. But it has limited antibacterial effect at "*in vivo*" condition owing to its inability to penetrate into the surrounding area of root canal such as apical anastomosis, lateral canals, and dentinal tubules. Moreover, the existence of non-activator materials (including exudates, collagen pulp tissue, and microbial

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populations) can interfere with the function of sodium hypochlorite and reduce its effectiveness, thereby making it impossible to obtain a canal system free from bacteria and pulp tissue.[4] It can penetrate into the dentinal tubules only up to 300 µm. Chlorhexidine is a wide-spectrum antimicrobial agent active against Gram-positive and gram-negative bacteria as well as yeasts. It is unique in its ability to bind to oral tissues for extended periods, from which it is released slowly (substantivity),[3] but it has less penetrative capacity.

The efficiency of irrigating solutions depends upon different factors such as mechanism of irrigation, its contact with potential substances, materials and root canal structures, their penetration depth in the main canal, and lateral spaces. However, none of the currently available irrigating solutions have all the properties needed. A combined use of different irrigants is therefore the recommended protocol to ensure successful clinical outcome. An *in vitro* study by Kuruvilla and Kamath concluded that when sodium hypochlorite and chlorhexidine solutions were combined within the root canal, the antibacterial action was suggestive of being augmented.[5]

Laser-activated irrigation aims at increasing irrigant activation and they have now become the latest choice in eradicating microorganisms in the root canal, especially in the lateral dentinal tubules. Many studies have concluded that the emission of laser light in the root canal does have an bactericidal effect. Gutknetch et al. analyzed the effects of the high power diode laser on extracted human teeth experimentally infected with *E. faecalis*. They reported the decrease of 99.9% bacteria after irradiation.[4,6] The Er,Cr:YSGG laser can remove calcified hard tissues by emitting a beam of infra-red energy at 2.78 µm, which works in combination with a water spray. This laser is highly absorbed by water from the surroundings and within the tissues and is therefore termed as the hydrokinetic system.[7,8]

Hence, the present study was carried out to compare and evaluate the antibacterial efficacy of different irrigant systems against *E. faecalis* and their horizontal depth of penetration into the dentinal tubules when used alone and when combined with lasers. The objective of this study was:

- To compare and evaluate the reduction in the colony count of bacteria with different irrigation systems, and
- To determine their horizontal depth of penetration into the dentinal tubules when used alone and in combination with lasers.

Materials and Methods

An experimental study *in vitro* was planned and conducted partly at the Department of Pedodontics and Preventive Dentistry, Royal Dental College, Palakkad and LASER4KIDS Practice, Chennai. Single-rooted teeth that were extracted for orthodontic and periodontal reasons were selected for the study. A total of 60 were collected, of which 42 were selected; those that were fractured, carious, and had resorption defects were excluded. The samples were stored in saline (0.9% w/v) (Fig. 1).

A 0.5 mm round diamond bur was used to prepare the access cavity in all the samples. The samples were then decoronated with the help of diamond disks. The apices of all these samples were sealed with wax. Root canals were prepared using Dentsply ProTaper rotary instruments and enlarged up to a file size of F2 of length 25 mm. Canals were irrigated with 3% sodium hypochlorite and 2% chlorhexidine gluconate and then with 15% EDTA to remove the smear layer. Postirrigation with sodium hypochlorite, the canals were thoroughly flushed with 0.9% normal saline and dried with absorbent points to prevent the formation of any precipitate before final irrigation with 2% chlorhexidine gluconate. The samples were sterilized by autoclaving after wrapping them with cotton rolls at 121°C at 15 minutes and 15 lbs pressure.

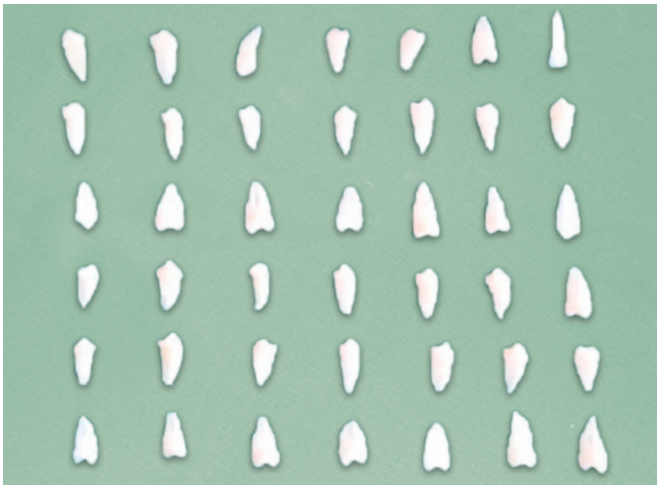


Fig. 1: Samples collected for the study

The strains used for this study were standard strains of *E. faecalis* ATCC 29212, which were subcultured in agar plate and incubated at 37°C for 24 hours (Fig. 2).

A sterile needle was used to inoculate the canals with 0.1 mL bacterial suspension in intact broth and this was incubated at 37°C for 24 hours. The colony counts in all the samples were determined (Fig. 3).

The samples were then randomly divided into six different groups, with each containing seven samples.

Study Groups

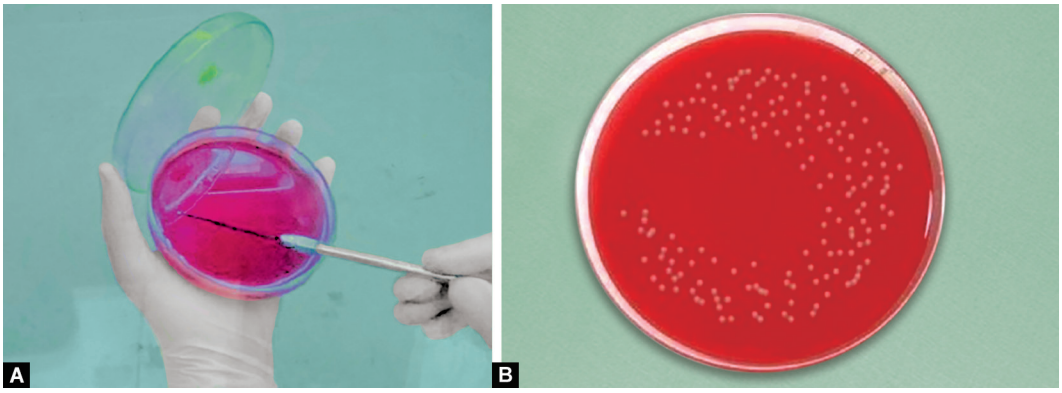
Group I

The samples in group I were treated with 0.9% normal saline for 10 seconds (Fig. 4).

Group II

The samples in group II were irradiated with diode laser (Zolar Diode, 810 nm, Canada) at a power output of 1 W in a continuous working mode at 1 mm/second for 10 seconds. This was repeated three times at an interval of 5 seconds (Fig. 5).

Fig. 2: ATCC 29212 strain of *Enterococcus faecalis*



Figs 3A and B: Determining the colony count of samples before disinfection

Fig. 4: Samples in group I

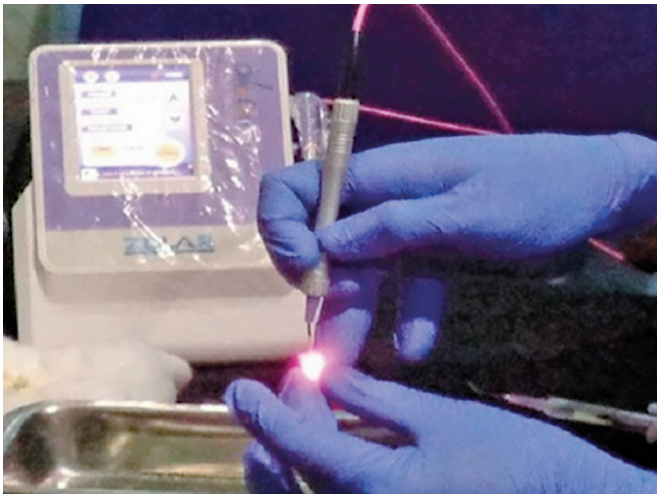
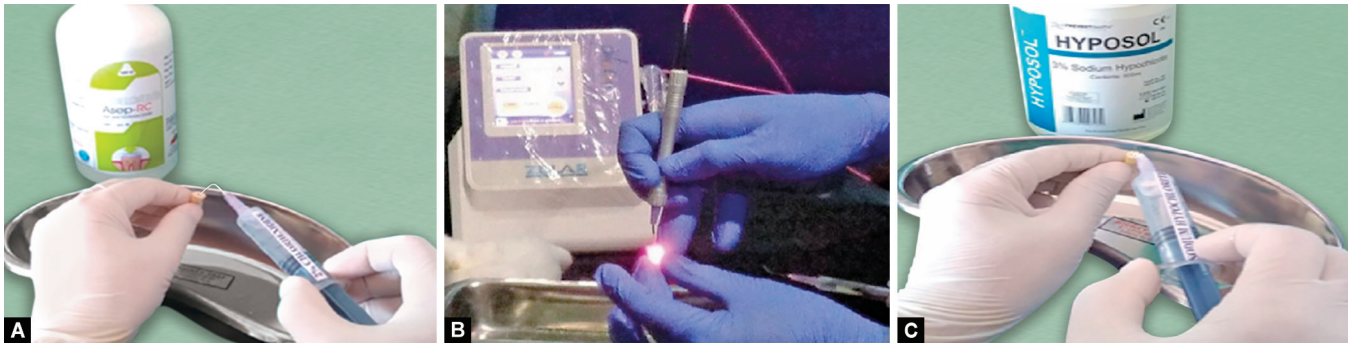


Fig. 5: Samples in group II



Figs 6A to C: Samples in group III

Group III

The samples in group III were treated with 3% sodium hypochlorite for 10 seconds, and then they were irradiated with diode laser for 10 seconds, where the same protocol was followed and repeated three times at an interval of 5 seconds. This was followed by irrigation with 0.9% normal saline for 10 seconds. They were then treated with 2% chlorhexidine gluconate for 10 seconds (Fig. 6).

Group IV

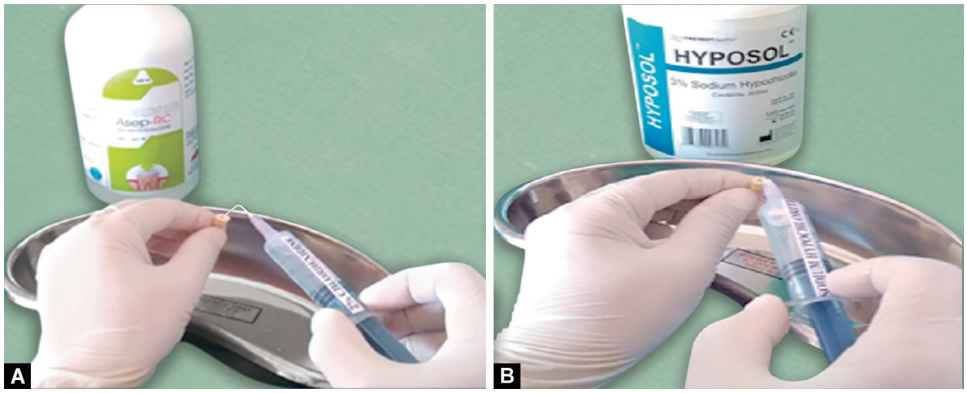
The samples in group IV were treated with 3% sodium hypochlorite for 10 seconds. They were irrigated with 0.9% normal saline for 10 seconds and then with 2% chlorhexidine gluconate for another 10 seconds (Fig. 7).

Group V

The samples in group V were irradiated with Er,Cr:YSGG laser (2780 nm, Waterlase MD®, USA) at 1 mm/second for 10 seconds. The fiber tip used was radial firing tip RFT2 at 0.75 W at 20 Hz frequency, 10% air, water off, and in hard tissue mode. This was repeated three times at 5-seconds interval (Fig. 8).

Group VI

The samples were initially treated with 3% sodium hypochlorite for 10 seconds. This was followed by Er,Cr:YSGG laser irradiation for 10 seconds, where the same protocol was followed, and was repeated three times at an interval of 5 seconds. They were irrigated



Figs 7A and B: Samples in group IV

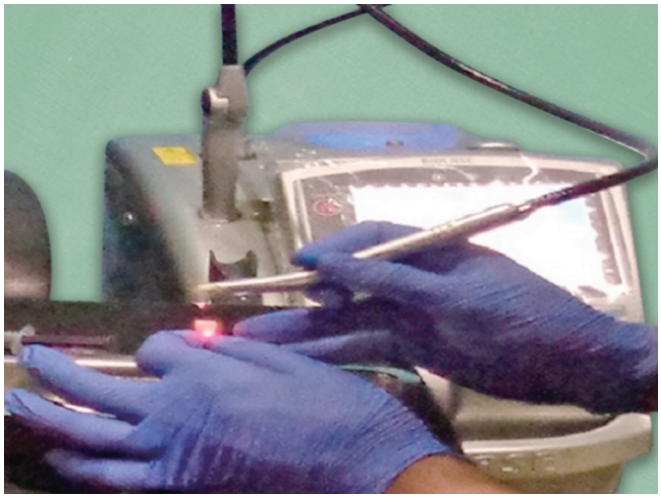


Fig. 8: Samples in group V

with 0.9% saline for 10 seconds followed by irrigation with 2% chlorhexidine gluconate for another 10 seconds (Fig. 9).

The colony count for all the samples after disinfection were determined manually by culture method (Fig. 10).

The samples were longitudinally sectioned using chisel and mallet and placed in crystal violet solution to observe the penetration depth under a stereomicroscope (20×). The width of the bleached zone was measured (in micrometers) and this was considered as the penetration depth (Fig. 11).

Results

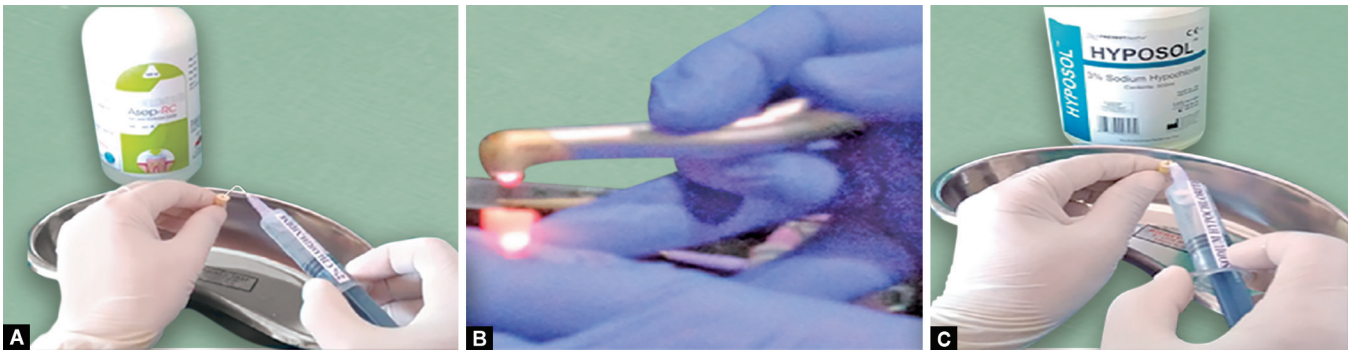
The CFU of bacteria for each group after disinfection are shown in Table 1. Group VI showed the least number of CFU and group I showed the highest. The results were highly significant ($p = 0.02^{a}$). The horizontal penetration depth of each group is shown in Table 2. Group VI was found to have the maximum penetration depth and group I showed the least penetration depth. The results were significant ($p = 0.01^{b}$).

Statistical Analysis

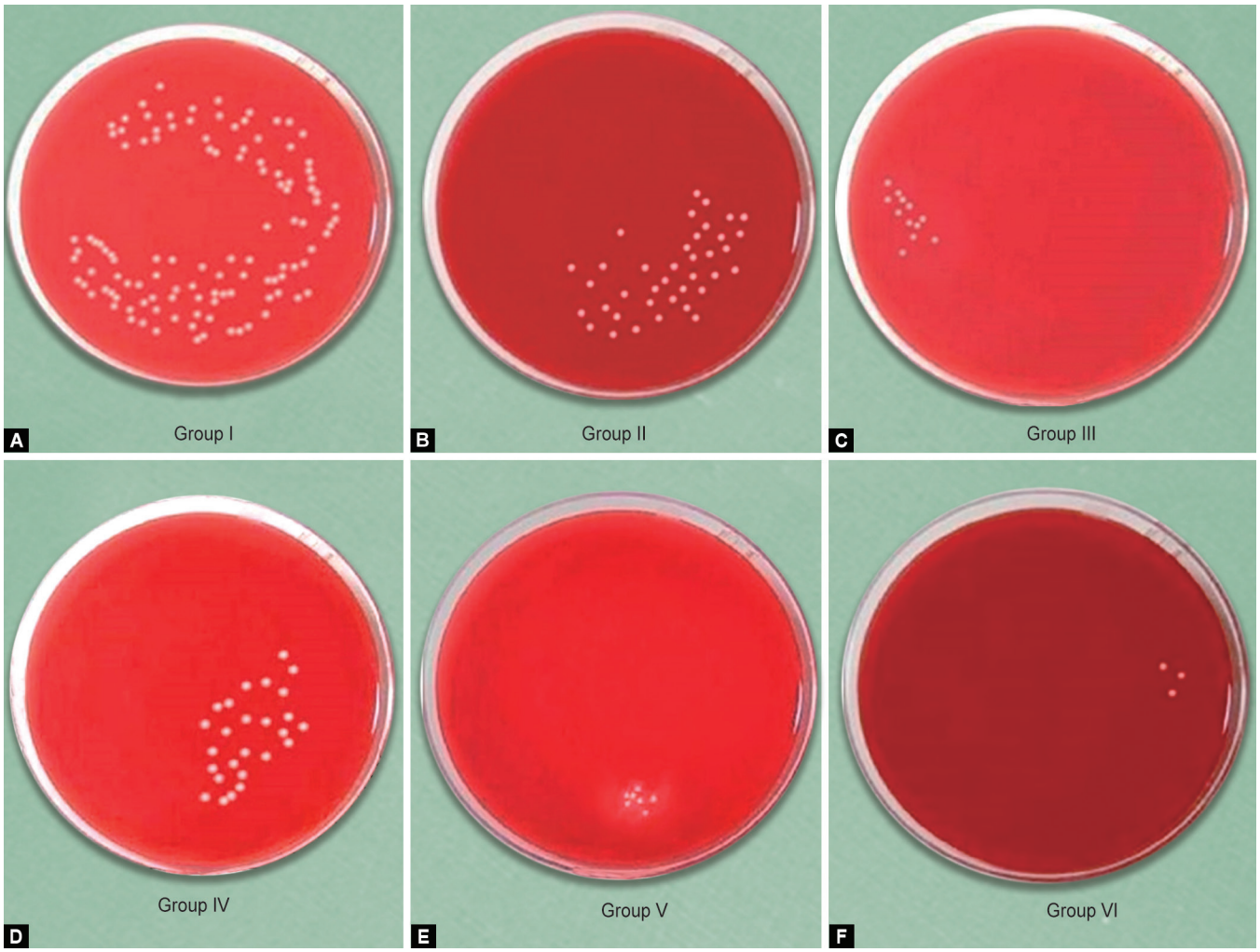
The data obtained were compiled systematically. The statistical analysis was done by an ANOVA test using a standard statistical package (SPSS Version 18.0 for Windows, SPSS Inc., Chicago, USA) (Figs 12 and 13). In all the groups, an intergroup comparison was done with Bonferroni *post hoc* (Table 3).

Discussion

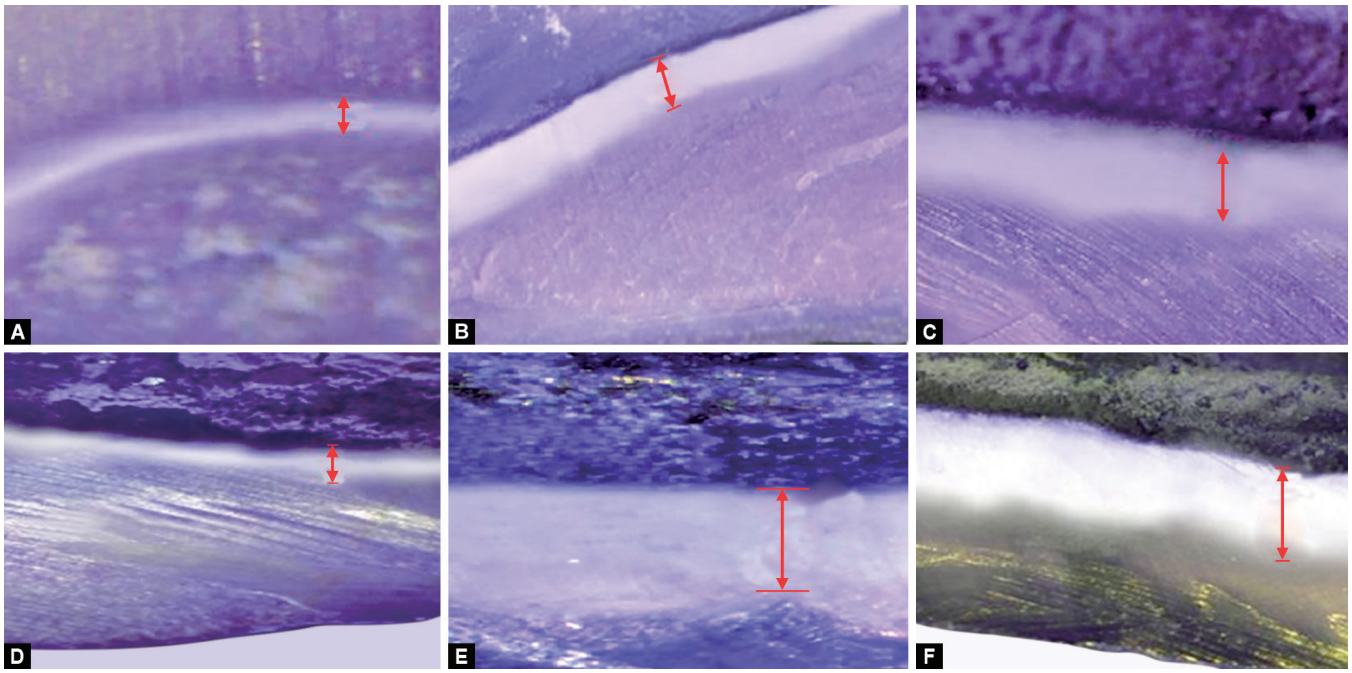
Several studies have shown that preparation of canals with manual and rotary devices whether stainless steel or nickel titanium cannot sufficiently disinfect root canals. Wang et al. in their study on the effects of diode laser on smear layer removal from canal walls and apical leakage after canal filling demonstrated that this laser is effective in debris and smear layer removal, leading to reduced apical leakage after filling.[9] Kangarloo and Fekrazad in a study investigated the effect of Er,Cr:YSGG laser on *E. faecalis* with 2% chlorhexidine solution in dentinal tubules of extracted teeth. Results showed that both laser and chlorhexidine solutions were effective in reducing bacterial count, but chlorhexidine was significantly more effective.[10] Gordan et al. studied the antimicrobial effect of Er,Cr:YSGG laser on dentinal walls infected by *E. faecalis* and reached the conclusion that Er,Cr:YSGG laser led to 99.7% reduction in bacterial count.[11] Schoop et al. assessed the effects of Er,Cr:YSGG laser on two types of microorganisms in root canals and stated that laser can eliminate intracanal bacteria.[12] Crystal violet was selected in this



Figs 9A to C: Samples in group VI



Figs 10A to F: Colony count of different samples after disinfection



Figs 11A to F: Penetration depth of different samples measured under stereomicroscope

Table 1: Colony-forming units of bacteria of various groups after disinfection

Groups	*N*	*Mean*	*Standard deviation*
Group I	7	11199.5	1.61
Group II	7	3799.7	0.9
Group III	7	1099.4	0.7
Group IV	7	2699.8	0.9
Group V	7	699	1.7
Group VI	7	299.5	0.7

Table 2: Horizontal depth of penetration (µm) of various groups

Groups	*N*	*Mean*	*Standard deviation*
Group I	7	60.1	0.1
Group II	7	241.3	1.6
Group III	7	350.5	0.3
Group IV	7	137.3	0.2
Group V	7	350.4	0.2
Group VI	7	450.3	0.2

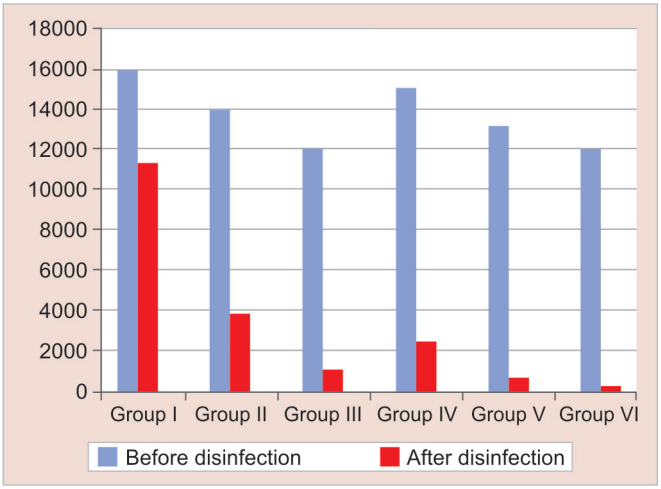


Fig. 12: A comparison of CFU of bacteria of different groups

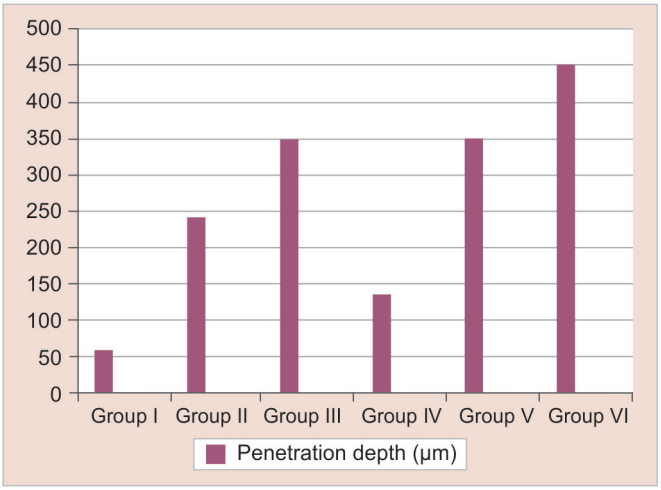


Fig. 13: A comparison of horizontal depth of penetration of different groups

Table 3: Multiple group comparison by Bonferroni *post hoc* test

Parameters	*p value*
CFU after disinfection between the groups	0.02[a]
Penetration depth (µm) between the groups	0.01[b]

[a,b]Significant difference amongst the groups ($p < 0.05$)

study for observation under a stereomicroscope because sodium hypochlorite is a strong oxidant. It whitens the purple color of crystal violet and reveals the clear natural color of dentin.[13] In the present study, when sodium hypochlorite was used in combination with Er,Cr:YSGG laser and chlorhexidine, there was a drastic reduction in the mean CFU of bacteria.

Thus, laser can be used directly or as an adjunctive device in disinfecting canals as it can penetrate the areas impossible with the traditional technique, where rinsing solutions have no access and eliminate microorganisms.[14] The present study shows that both diode laser and Er,Cr:YSGG laser are effective in eradicating microorganisms, but more successful when the latter is used along with 3% sodium hypochlorite and 2% chlorhexidine gluconate.

Conclusion

In this study, the CFU of each group before and after disinfection as well as the horizontal depth of penetration were determined. Thus, it can be concluded that Er,Cr:YSGG laser in combination with 3% sodium hypochlorite and 2% chlorhexidine gluconate can be used to disinfect the root canal system.

Clinical Significance

This study highlights that Er,Cr:YSGG laser was efficient enough to bring about maximum reduction in the bacterial colony count when used in combination with other irrigants. Moreover, it showed the greatest depth of penetration when compared to the rest of the groups. Hence, laser-assisted irrigation regime can be employed in clinical practice for providing a successful endodontic therapy.

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