Comparative Evaluation of Bactericidal Effects on *Enterococcus faecalis* Using Diode Laser Irradiation, Sodium Hypochlorite and Chlorhexidine Gluconate Irrigation"- an *In vitro* Study

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Abstract

Aim: This *in vitro* study was performed to evaluate the bactericidal effects of Diode laser, 3% Sodium hypochlorite and 2% Chlorhexidine gluconate irrigation on root canals infected with *Enterococcus faecalis*.

Method: Seventy single rooted central incisors were prepared and sixty was contaminated with *Enterococcus faecalis*. After 48 hours of incubation samples were divided into 7 groups-Group 1- Sterility control (canals not contaminated with *Enterococcus faecalis*); Group 2- Positive control canals with no other treatment; Group 3- canals were treated with 3% NaOCl during biomechanical preparation; Group 4- canals were treated with 2% CHX; Group 5- canals were treated with 980nm diode laser; Group 6- was treated with combination of laser and 3% NaOCl; Group 7- were treated with laser and 2% CHX, the CFU of all the groups were checked.

Result: Group 3 and 6 were significantly effective as there was no growth of *E. faecalis* (p=<0.001), group 7 was significantly more effective than group 2 (p=<0.001). There was no statistical significance between group 7 and group 3 and 6 (p=0. 474), the maximum amount of growth was seen in group 4.

Conclusion: 3% NaOCl was the most effective irritant comparatively and combination of 2% CHX and laser was as effective as 3% NaOCl and hence can be used as an alternative for 3% NaOCl.

Key Words: Diode Laser, Chlorhexidine, Sodium Hypochlorite

Introduction

Obtaining a root canal system free of irritants is a major goal of root canal treatment because remains micro-organisms may cause persistent inflammation in the periradicular tissues [1,2].

It has been reported that viable bacteria remain within the canal system even after mechanical preparation [3]. These observations call for an effective intracranial disinfection of the root canal system. The effectiveness of intracanal medications in achieving disinfection is still controversial. Tissue toxicity seems to be directly related to its antimicrobial effects. The most potent antimicrobial medications are also the most irritating to the host tissues [4].

Sodium hypochlorite, a clear straw-colored reducing agent, in concentrations ranging from 0.5% to 5.25% is currently one of the most commonly used reagents in endodontic therapy. Its antimicrobial and tissue-dissolving properties have been widely reported. In addition, it is inexpensive, has a long shelf-life, and is readily available. Unfortunately, the use of NaOCl does not come without reservations. Its extreme cytotoxicity elicits severe inflammatory tissue reaction, when accidentally injected beyond the root apex, followed by extreme pain, edema, and hematoma [4-6].

Chlorhexidine gluconate solutions in varying concentrations of (0.2-2%) have been recommended as an endodontic irrigant. Its broad spectrum activity against Gram-positive and Gram-negative bacteria, its ability to adsorb to dental tissues and mucous membrane with prolonged gradual release at therapeutic levels, as well as its biocompatibility are some of the properties that justify its clinical use [4-6].

Enterococcus faecalis, is a facultative Gram-positive anaerobic coccus that is a known endodontic pathogen. It has been frequently recovered from the root canals of teeth associated post-treatment diseases and persistently causes root canal infection. It is also resistant to interappointment medicaments, including calcium hydroxide. It may also reside in the canals as single species without the support of other microorganisms. These microorganisms under specific conditions have the ability to infect the whole length of tubules within few days. Hence it's of prime importance to eradicate these bacteria from the root canal [2].

With the introduction of lasers to the field of conservative dentistry and endodontic treatment has been enriched by a multitude of new treatment methods that improved the chances for a successful treatment outcome.

With the great progresses in the field of laser technology, semiconductor lasers such as diode laser are gaining increasing importance. The diode laser can achieve an output power of several watts and shown to be highly reliable and effective; the diode laser can be recommended for endodontic treatment because its wavelength of 980nm which is within the infrared range, it also has thin, flexible light-conductor fiber [7-9].

Medicaments or irritants used in combination or in succession have been reported to be effective for canal disinfection [10].

Objective

Aim

In this *in vitro* study, we investigated the bactericidal effect of 980 nm diode laser and irrigating solutions such as 3% sodium hypochlorite and 2% chlorhexidine and their combination with laser in *E. faecalis* infected root canals.

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Methods and materials

70 extracted single rooted teeth with fully developed apices were collected from the Department of Oral and maxillofacial surgery, A. B. Shetty Memorial Institute of Dental Sciences, NITTE University, Mangalore. The teeth used for study purposes were obtained from the patients after receiving their consent. The teeth were disinfected as per Occupational Safety and Health Act (OSHA) / Centre of Diseases Control (CDC) regulations and cleaned from attachment debris and calculus, and used [3]. The crowns were then removed 2 mm above the cemento-enamel junction by using a high-speed diamond bur (SS White®Burs, USA, and ISO 9001) with water coolant. Working length will be determined with the help of the Dental radiovisiography (Intra Skan. DC, SKANRAY Technologies, Mysore, India). The roots would be prepared by serial preparation to #30 k-file (DENTSPLY Maillefer, China), with saline as irrigant, for easier inoculation of bacteria. The preparation would be stopped at the apical constriction. Two coats of nail Varnish are applied to seal the apex. The canals have to be dried with sterile paper points (DENTSPLY Maillefer, China) and sterilization achieved using an Autoclave at 121°C (MELAG Medizintechnik Geneststraβe, Berlin, Germany) for 15 min with teeth immersed in distilled water [2].

Selection and preparation of bacteria: A pure bacterial culture of the Gram-positive cocci *Enterococcus faecalis* (ATCC 10541) [Hi Media Laboratories, Mumbai, India] was obtained. The grown bacterial colonies were then harvested and placed in Mueller-Hinton nutrient broth (Hi Media Laboratories, Mumbai, India) and incubated for 24 hrs at 37°C under aerobic conditions. The turbidity of Mueller-Hinton broth containing *E. faecalis* was adjusted to McFarl and 0.5 standard which corresponds to 1.5×10^8 colonies. Then the 20 µL of the bacterial culture was transferred into the canal lumen of the mechanically enlarged root canals using a sterile micropipette and stored at 48 hrs at 37°C.

The selected prepared teeth were randomly divided into 7 groups (ten teeth for each group) (*Table 1*):

Group 1: Sterility control here canals are not contaminated with *E. faecalis* this is done to evaluate that there should be no contamination of the canals by other micro-organism.

Group 2: positive control here the canals is inoculated with *E. faecalis* and were not treated with any irritant

Group 3: In these samples between each step of biomechanical preparation, 3% NaOCI will be used as an irritant.

Group 4: In these samples between each step of biomechanical preparation, 2% CHX will be used as an irritant.

Group 5: Here after the biomechanical preparation 980 NM Diode laser (KaVo GENTLEray 980 Classic Plus, Switzerland) is used with a fiber tip of 200 µm with the power of 2.5 watts in continuous mode. The fiber tip was inserted into the root canal at a distance of 1mm from the apical foramen and moved from apex to coronal region in consecutive circular motion with 3 cycles each cycle of 5 secs [4].

Table 1. *Description of the various study groups.*

Groups	Group Description
Group I	Sterility Control Group
Group II	Positive Control Group
Group III	3% NaOCl Group
Group IV	2% CHX Group
Group V	980 nm Diode Laser Group
Group VI	3% NaOCl + 980 nm Diode Laser Group
Group VII	2% CHX + 980 nm Diode Laser Group

Group 6: Here between each step of preparation, 3% NaOCI (Vensons Pvt. Ltd, Bangalore, India) will be used as irrigant. And 980 nm diode laser will be used after preparation as used in group 5.

Group 7: Here between each step of preparation, 2% CHX (Vishal Dentocare Pvt. Ltd, Nashik, India) will be used as irrigant. And 980 nm diode laser will be used after preparation as used in group 5.

The experimental teeth were then irrigated with 1 ml of normal saline solution. The saline solution from the canals was collected in a sterile test tube. Samples from test tubes showing turbidity were streaked with nichrome wire loops (4 mm) on agar plates and incubated at 37°C for 48 h, for the confirmation of Enterococcus colony growth). Data obtained were tabulated and analyzed statistically for differences using chi-squared test, comparing pairs of groups, with a significance level established at $P<0.05$.

Results

The comparisons between all the groups is shows there is a significant difference between the control groups and the test groups (*Tables 2* and *3*) (p=<0.001). In Group 1, no *E. faecalis* was inoculated and there was no contamination. Group 3 and 6 were significantly effective as there was no growth of *E. faecalis* compared to group 4 and 5 (p=<0.001) (*Tables 4* and *5*; *Figure 1*), group 7 was significantly more effective than group 4 and group 5 (p=<0.001) (*Tables 6* and *7*; *Figure 2*). There was no statistical significance between group 7 and group 3, 6 (p=0.474), maximum amount of growth was seen in group 2 (*Tables 8* and *9*; *Figure 3*).

Discussion

Microorganisms in the root canals have long been recognized as the primary etiologic factors in the development of pulp and periapical lesions [11]. Elimination of the micro-organisms is one of the important objectives for successful root canal treatment. It is necessary to chemically Debride teeth with complex internal anatomy or other irregularities that might be missed by the instrumentation of the root canals [12]. Hence the use of irrigants during root canal treatment is of prime importance.

The use of irrigating solutions is basically used for removal of tissue debris and microorganisms and prevents formation of apical plug that can block the root canal which is produced during instrumentation [13]. It is also used for removal of smear layer and increase cutting efficiency of the instruments [14]. The ideal requirement of irrigating solution was: dissolving action, germicidal effect and non irritating to the periapical tissue. Unfortunately, no single irrigant fulfills all of these criteria [15].

E. faecalis (ATCC 10541) was selected as the test organism because it is commonly associated with root canal failure cases and persistent apical periodontitis. It is also resistant interappointment calcium hydroxide dressing [2]. They also have the ability to reside in the canals without the support of other micro-organisms and under specific conditions have the ability to infect the whole length of tubules within few days.

In the present study 3% NaOCl is used as an irritant that showed no growth of *E. faecalis* in the canals, it is a broad-spectrum antimicrobial agent. It has powerful oxidative activity not only dissolves the papal and dentinal tissue but also flushes them out of the canals [16]. Even though being one of the most widely used

Table 2. *Description of the various study groups.*

			Groups							Total
			Positive Control	Negative Control	CHX	NaOCl	Laser	CHX + Laser	NaOCl + Laser	
Growth	NG	Count	0	10	0	10	0	8	10	38
		% within Growth	0.0%	26.3%	0.0%	26.3%	0.0%	21.1%	26.3%	100.0%
		% within group	0.0%	100.0%	0.0%	100.0%	0.0%	80.0%	100.0%	54.3%
	$< 10^3$	Count	0	0	2	0	10	2	0	14
		% within Growth	0.0%	0.0%	14.3%	0.0%	71.4%	14.3%	0.0%	100.0%
		% within group	0.0%	0.0%	20.0%	0.0%	100.0%	20.0%	0.0%	20.0%
	10^3	Count	0	0	8	0	0	0	0	8
		% within Growth	0.0%	0.0%	100.0%	0.0%	0.0%	0.0%	0.0%	100.0%
		% within group	0.0%	0.0%	80.0%	0.0%	0.0%	0.0%	0.0%	11.4%
	10^5	Count	10	0	0	0	0	0	0	10
		% within Growth	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	100.0%
		% within group	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	14.3%
Total		Count	10	10	10	10	10	10	10	70
		% within Growth	14.3%	14.3%	14.3%	14.3%	14.3%	14.3%	14.3%	100.0%
		% within group	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

(NG- no growth, CFU- colony forming units, CHX- chlorhexidine, NaOCl- sodium hypochlorite, LA- laser)

Table 3. *Chi-Square Tests (p value).*

	Value	Exact Sig. (2-sided)
Fisher's Exact Test	107.236	.000
No of Valid Cases	70	

Table 4. *Cross tabulation of CFU in various groups.*

			Colony			Total
			NG	<1000	>=1000	
Group	CHX	**Count**	**0**	**2**	**8**	**10**
		% within Group	0.0%	20.0%	80.0%	100.0%
		% within Colony	0.0%	16.7%	100.0%	33.3%
	NaOCl	Count	10	0	0	10
		% within Group	100.0%	0.0%	0.0%	100.0%
		% within Colony	100.0%	0.0%	0.0%	33.3%
	LA	Count	0	10	0	10
		% within Group	0.0%	100.0%	0.0%	100.0%
		% within Colony	0.0%	83.3%	0.0%	33.3%
Total		Count	10	12	8	30
		% within Group	33.3%	40.0%	26.7%	100.0%
		% within Colony	100.0%	100.0%	100.0%	100.0%

(NG- no growth, CFU- colony forming units, CHX- chlorhexidine,NaOCl- sodium hypochlorite, LA- laser)

reagents in endodontic therapy but its toxicity to periapical tissues remains a principle concern [17]. Hence there is a need of irrigant which could be used as a substitute to sodium hypochlorite having similar advantages and lesser cytotoxic effects.

Chlorhexidine is used in various concentrations (0.002-2%), they have the ability to bind to the hydroxyapatite components and gradually releases bound chlorhexidine hence they protect against the microbial colonization beyond the intended period [5,17]. As per the results, 2% CHX solution was efficient in partially disinfecting the canals hence cannot be suggested as sole root canal irrigant.

Recently lasers have shown great promise in the field of Endodontics also in the removal of smear layer in instrumented root canals. Studies have demonstrated the bactericidal effects of diode laser are very promising in root canals [18,19]. Moritz et al. reported that an 809-nm diode laser was able to clean the root

Table 5. *Chi-Square Tests (p value).*

	Value	Exact Sig. (2-sided)
Fisher's Exact Test	42.619	.<0.001
No of Valid Cases	30	

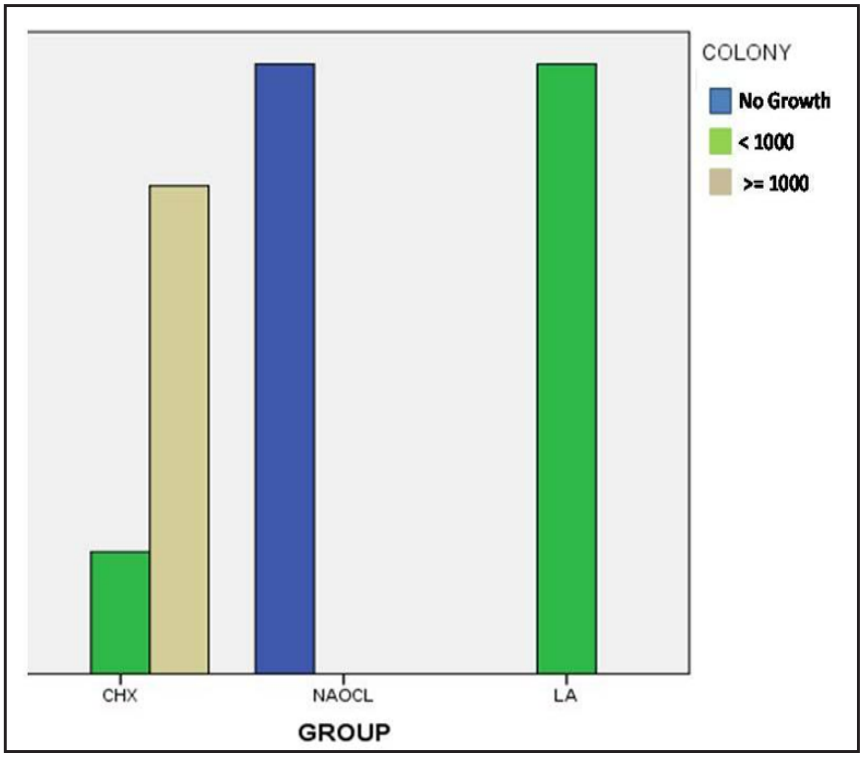

Figure 1. *Group wise comparisons of the growth of E. faecalis in Group 3, 4 and 5.*

Table 6. *Cross tabulation of CFU in various groups.*

			Colony			Total
			NG	<1000	>=1000	
Group	CHX	Count	0	2	8	10
		% within GROUP	0.0%	20.0%	80.0%	100.0%
		% within COLONY	0.0%	50.0%	100.0%	50.0%
	CHX+LA	Count	8	2	0	10
		% within GROUP	80.0%	20.0%	0.0%	100.0%
		% within COLONY	100.0%	50.0%	0.0%	50.0%
Total		Count	8	4	8	20
		% within GROUP	40.0%	20.0%	40.0%	100.0%
		% within COLONY	100.0%	100.0%	100.0%	100.0%

(NG- no growth, CHX- chlorhexidine, CHX+LA- chlorhexidine in combination with laser)

Table 7: *Chi-Square Tests (p value).*

	Value	Exact Sig. (2-sided)
Fisher's Exact Test	17.217	<0.001
No of Valid Cases	20	

canal wall as well as close the dentinal tubules [13].

In this study 980 nm diode laser is used, the antibacterial action of the diode laser is not as effective as 3% NaOCl but is significantly more effect compared to 2% CHX. The inability of the diode laser to completely kill the bacteria could attribute to the penetration of *E. faecalis* deep into the dentinal tubules because of the removal of smear layer prior to contamination [20]. The delivery of energy of the laser beam is only when it is focused due to this it may not be possible to direct the beam over the entire root canal surface. Another factor that could prevent complete elimination of *E. faecalis* could be the anatomy of the root canals.

This problem could be solved with the use of combination between laser and irrigants. The combination of diode laser and 2% CHX have proved to be significantly effective compared to the use of laser and chlorhexidine alone. A combined regimen of CHX with laser irradiation was found to be a potentially very effective in inhibition of bacterial growth [20].

In this study there was no significant difference is the effect between 3% NaOCl and the combination of 980 nm diode laser and 2% CHX hence a combination of 2% CHX and diode laser can be used as an alternative to 3% NaOCl for disinfection of root canals to prevent from hypochlorite accident and in cases where NaOCl is contraindicated such as cases with open apex and blunderbuss canals and in patients with NaOCl allergy [21,22].

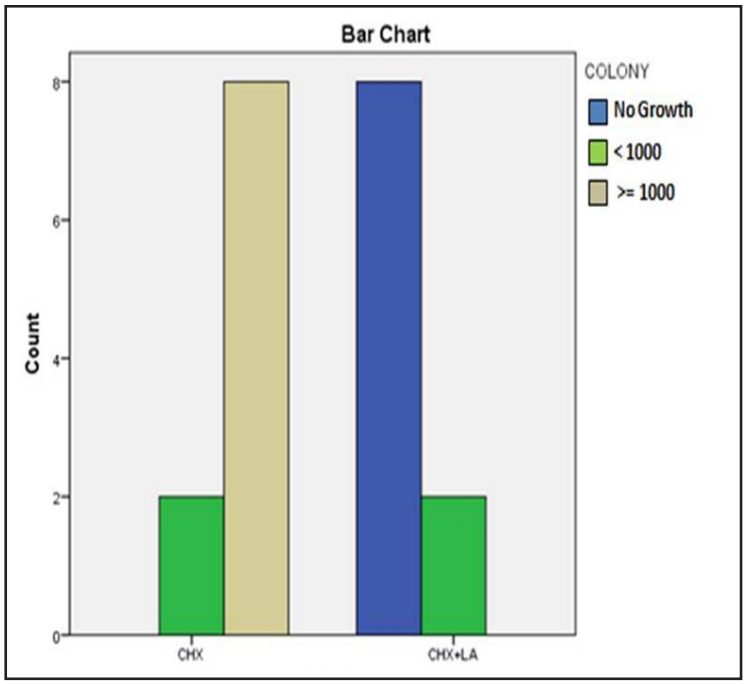

Figure 2. *Group Wise comparisons of the growth of E. faecalis in group 4 and 7.*

Table 8. *Cross tabulation of CFU in various groups.*

			Group		Total
			NaOCL	CHX+ LASER	
Growth	NG	**Count**	**10**	**8**	**18**
		% within growth	55.6%	44.4%	100.0%
		% within group	100.0%	80.0%	90.0%
	<1000	Count	0	2	2
		% within growth	0.0%	100.0%	100.0%
		% within group	0.0%	20.0%	10.0%
Total		Count	10	10	20
		% within growth	50.0%	50.0%	100.0%
		% within group	100.0%	100.0%	100.0%

Table 9. *Chi-Square Tests (p value).*

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)
Pearson Chi-Square	2.222[a]	1	.136	.474
N of Valid Cases	20			

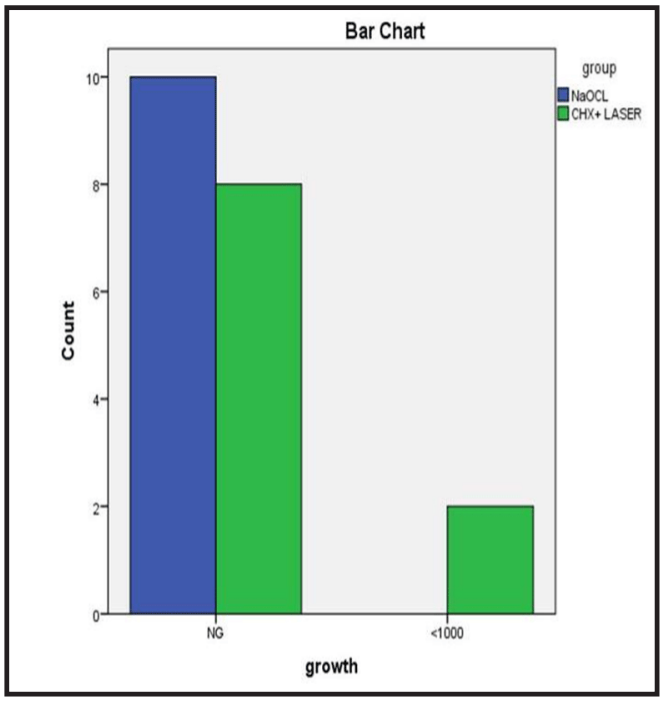

Figure 3. *Group Wise comparisons of the growth of E. faecalis in group 3 and 7.*

Conclusion

From the results of this study it could be concluded that 3% NaOCl was the most effective irrigant for disinfection of root canals with *E. faecalis*. 980 diode laser was significantly more effective than 2% CHX and combination of 2% CHX and laser was significantly more effective than laser alone and was as effective as 3% NaOCl and hence can be used as an alternative for 3% NaOCl.

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- MH, KS and SS participated in the design, performing the examinations, coordination of the study, and drafted the manuscript.

- MH, SS participated in the design, coordination and supervision of the study.

- MH, SS and MK Jr. participated in the statistical analysis.

- VS and KS participated in the Microbiological analysis.

- All authors participated in the design, coordination and supervision of the study and drafted the manuscript.

- All authors read and approved the final manuscript.

Conflict of Interest

No conflict of Interests reported.

References

1. Blum J-Y, Michailesco P, Abadie M. An evaluation of the Bactericidal effect of the Nd: YAP Laser. *Journal of Endodontics* 1997; **23**: 583-585.

2. Eldeniz A.U, Ozer F, Hadimli HH. Bactericidal efficacy of Er, Cr: YSGG laser irradiation against *Enterococcus faecalis* compared with NaOCI irrigation: an ex vivo pilot study. *International Endodontic Journal* 2007; **40**: 112-119.

3. Guidelines for Infection Control in Dental Health Care Settings --- CDC guidelines 2003. Accessed (2012 March 22) at: www.cdc.gov/oralhealth/infectioncontrol/guidelines/index.htm

4. Bergmans L, Moisiadis P, Teughels W. Bactericidal effect of Nd:YAG laser irradiation on some endodontic pathogens ex vivo. *International Journal of Endodontics* 2006; **39**: 547-557.

5. Karale R, Thakore A, Shetty VK. An evaluation of antibacterial efficacy of 3% sodium hypochlorite, high-frequency alternating current and 2% chlorhexidine on *Enterococcus faecalis*: An *in vitro* study. *Journal of Conservative Dentistry* 2011; **14**: 2-5.

6. Izu KH, Thomas SJ, Zhang P. Effectiveness of sodium hypochlorite in preventing inoculation of periapical tissues with contaminated patency files. *Journal of Endodontics* 2004; **30**: 92–94.

7. White RR, Janer LR, Hays GL. Residual antimicrobial activity associated with a chlorhexidine endodontic irrigant used with sodium hypochlorite. *American Journal of Dentistry* 1999; **12**: 148–150.

8. Gutknecht N. State of the Art in lasers for dentistry. Journal of Laser and Health Academy 2008; **3**: 1-5.

9. Gutknecht N. Lasers in endodontics. *Journal of Laser and Health Academy* 2008; **4**: 1-5.

10. El-Batanouny MH. Electron microscopic study on the effect of diode laser and some irrigants on root canal dentinal wall. *Cairo Dental Journal* 2008; **24**: 421:427.

11. Wang X, Sun Y. Effects of Diode Laser Irradiation on Smear Layer Removal from Root Canal Walls and Apical Leakage after Obturation. *Photomedicine and Laser Surgery* 2007; **23**: 575-581.

12. Chang Y-C, Huang F-M. The effect of sodium hypochlorite and chlorhexidine on cultured human periodontal ligament cells. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontology Journal* 2001; **92**: 446-450.

13. Moritz A, Gutknecht N, Goharkhay K. *In vitro* irradiation of infected root canals with a diode laser: results of microbiologic, infrared spectrometric, and stain penetration examinations. *Quintessence International* 1997; **28**: 205.

14. Gutknecht N, Franzen R, Schippers M. Bactericidal effect of 980-nm diode laser in the root canal wall dentin of bovine teeth. *Journal of Clinical Laser Medicine Surgery* 2004; **22**: 9–13.

15. Sjogren U, Figdor D, Persson S. Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. *International Journal of Endodontics* 1997; **30**: 297-306.

16. Kandaswamy D, Venkateshbabu N. Root canal irrigant. *Journal of Conservative Dentistry* 2010; **13**: 256-264.

17. White RR, Hays GL, Janer LR. Residual Antimicrobial Activity after canal Irrigation with Chlorhexidine. *Journal of Endodontics* 1997; **23**: 229-231.

18. Zehnder M. Root Canal Irrigants. *Journal of Endodontics* 2006; **32**: 389-398.

19. Sahar-Helft S, Slutzky-Goldberg I. Synergistic Effect of Er: YAG Laser Irradiation in Combination with Chlorhexidine on the Viability of *Enterococcus faecalis*: An *in vitro* Study. *Photomedicine and Laser Surgery* 2011; **29**: 753–758.

20. Asnaashari M, Asnaashari N. Clinical Application of 810 nm Diode Laser and Low Level Laser Therapy for Treating an Endodontic Problem: A Case Presentation. *Journal of Lasers Medical Science* 2011; **2**: 82-86.

21. Hulsmann M, Hahn W. Complications during root canal irrigation- literature review and case reports. *International Endodontic Journal* 2000; **33**: 186-193.

22. Mehrvarzfar P, Saghiri MA. Additive effect of a diode laser on the antibacterial activity of 2.5% NaOCl, 2% CHX and MTAD against *Enterococcus faecalis* containing root canals: an *in vitro* study. *Journal of Oral Science*. 2011; **53**: 355-360.