Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 1, 2021, Pages. 6227 - 6236
Received 15 December 2020; Accepted 05 January 2021.

Comparison of Efficacy of 2.5% Vs 5.25% Concentrations of Sodium Hypochlorite Augmented by Different Irrigation Techniques in Eradication of Enterococcus Faecalis – An In-Vitro Study

Rupam Kaur[1], Kanwalpreet Kaur Bhullar[2], Deepali[3], Shantun Malhotra[4], Aashish Handa[5]

[1,4,5]Reader, Department of Conservative Dentistry and Endodontics, Sri Guru Ramdas Institute of Dental Sciences and Research, Amritsar, Punjab, India;

[2]Professor and Head of the Department, Department of Conservative Dentistry and Endodontics, Sri Guru Ramdas Institute of Dental Sciences and Research, Amritsar, Punjab, India;

[3]Post graduate student, Department of Conservative Dentistry and Endodontics, Sri Guru Ramdas Institute of Dental Sciences and Research, Amritsar, Punjab, India

Corresponding author:

Dr.Deepali, Post graduate student, Department of Conservative Dentistry and Endodontics, Sri Guru Ramdas Institute of Dental Sciences and Research, Amritsar, Punjab, India; E mail: deepalimahajan003@gmail.com

ABSTRACT:

Background: Enterococcus faecalis has been widely used as a bacteria in in-vitro studies to access the antibacterial efficacy of irrigants and various techniques of activation. The aim of the present study is to determine the efficacy of 2.5% and 5.25% sodium hypochlorite when both the concentrations are augmented with passive ultrasonic irrigation and lasers and comparing it with conventional syringe irrigation in eradication of E. fecalis.

Materials and method: A total of sixty single rooted teeth were included. Access cavities were made, root canals were instrumented till size 40 K file andpure culture of Enterococcus faecalis (ATCC 29212) was used to contaminate the root canals. Teeth were divided into 3 groups according to the technique used: Group I – irrigation with conventional syringe/needle method; Group II – ultrasonic activated irrigation; Group III – Laser activated irrigation using 810 nm diode laser. All the groups were further divided into two subgroups according to concentration of NaOCl used: Subgroup A – 2.5%; Subgroup B - 5.25%. The colony forming units were measured for all the samples and analysed.

Results: The results of one-way analysis of variance (ANOVA) showed that mean value of Group-I (223.06), Group-II (7.53) and Group-III (207.24) differ significantly from each other. The maximum eradication was seen with 5.25% NaOCl potentiated with ultrasonics (CFU – 0.00) and the minimum eradication was seen with 2.5% NaOCl delivered using conventional syringe (431.78). Mean value of colony forming units with 2.5% concentration and ultrasonics (15.06) was statistically comparable with 5.25% concentration and conventional syringe (14.34).

Conclusion: Ultrasonic activated irrigation exhibited maximum efficacy followed by lasers and conventional irrigation method. 5.25% NaOCl showed more efficacy compared to 2.5%. Efficacy of 2.5% NaOCl can be increased by ultrasonic activation and can be used as an alternative to 5.25% concentration to avoid the caustic effects of higher concentration.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 1, 2021, Pages. 6227 - 6236
Received 15 December 2020; Accepted 05 January 2021.

Keywords: Enterococcus faecalis, conventional needle irrigation, ultrasonics, diode laser, sodium hypochlorite.

INTRODUCTION

The successful outcome of root canal treatment is based on efficient disinfection of the root canal system and prevention of reinfection. The goal of treatment should not only be complete removal of microorganisms but also to prevent any damage to periapical tissues from treatment procedures so as to provide a conducive envoirnment for healing of periapical areas.

One of the main reason for failure is the re-infection of the root canals with microorganisms. Enterococcus fecalis is a facultative anaerobe, gram positive coccus, which is present in oral flora and is the most common strain isolated from teeth with endodontic treatment failure (Love RM et al., 2001[1], Zhang C, 2015[2]) and persistent apical periodontitis (Sundqvist G et al., 1998)[3]. It is difficult to eradicate this bacteria by traditional techniques because of its capability to form biofilm.

Irrigation is an essential part of root canal debridement to achieve intracanal disinfection beyond what might be achieved by root canal instrumentation alone (Gulabivala K et al., 2010)[4]. It was seen that only 40% of the root canal walls in the apical area of oval canals can be contacted by rotating instruments[5]. Therefore, irrigation and chemical debridement are essential as it allows for cleaning beyond the root canal instruments. Bystrom and Sunqvist[6] found that the use of irrigant in addition to mechanical preparation lowered the bacterial count to 40-60%.

NaOCl has been regarded as gold standard among irrigants and is the most commonly used endodontic irrigant (Sohrabi K et al., 2016)[7]. It is used in concentration ranging from 0.5 to 5.25%.Reviewing literature it can be stated that NaOCl is associated with a dose-dependent antimicrobial and tissue dissolving effect that increases with higher concentrations, however, it is accompanied higher cytotoxicity and liability to extrude periapically (Zehnder 2006[8], Boutsioukis et al. 2013[9]).It was found (study) that at high concentration (5.25%) severe irritations have been reported after inadvertent extrusion into periapical tissues (Arun J et al.,2017[10]).In a study conducted by Mostafa MEHAA et al. (2020)[11], it was found that the use of 5.25% NaOCl caused more pain incidence and intensity than 1.3%% NaOCl.

The above studies suggest the need for a better clinical alternative to potentiate the effect of lower concentrations of NaOCl. Different agitation techniques have been proposed to enhance the flushing action in order to increase the efficacy of irrigation (Gu LS et al., 2009[12], Stojicic S et al. 2010[13]).

Therefore the aim of this study was to determine the efficacy of 2.5 % Naocl with 5.25% when both irrigants are augmented with passive ultrasonic irrigation and lasers and comparing it with conventional irrigation in eradication of E. fecalis.

MATERIALS AND METHODS

This research was conducted in the Department of Conservative Dentistry and Endodontics of Sri Guru Ram Das Institute of Dental Sciences and Research in collaboration with the Department of Microbiology of SGRD medical college, Amritsar.

This study was performed on sixty freshly extracted single rooted teeth. They were washed and cleaned with ultrasonic scalers to remove any debris. Conventional access to the root canal system was performed. Patency of each canal was established by placing a size 10 K file until it was visible in the apical foramen. Working length was established 1 mm short of the apex, and the canals were enlarged sequentially upto 40 K file. EDTA was used as a lubricant and canals were irrigated using 5.25% NaOCl solution during the preparation.

After root canal preparation, the enlarged apical foramen was sealed using acrylic resin to prevent bacterial leakage. The specimens were then sterilized in an autoclave at 121 °C for 15 minutes at 15 psi pressure.

Pure culture of Enterococcus faecalis (ATCC 29212) grown in brain heart infusion (BHI) broth, was used to contaminate the root canals. The root canals were inoculated with 15 µm of the turbid suspension of Enterococcus faecalis using a micropipette. The turbidity was verified using the McFarland turbidity scale, and adjusted to 0.5 corresponding to 10^8 organisms per millilitre. The specimens were incubated at 37 degrees for 24 hours.

Colony forming units (CFUs) of E. faecalis was counted for one sample in each group to ensure growth of root canals. Asepsis was maintained throughout the procedures during standard precautions.

The teeth were randomly divided into three groups of 20 each:-

GROUP 1 – Conventional irrigation using needle/syringe with sodium hypochlorite solution.

After incubation, samples were retrieved from the incubator. The canals were subjected to copious syringe irrigation with 10 ml of NaOCl solution and 2 ml of 17% EDTA alternately for 1 min. Finally, canal was washed with 2 ml of saline to remove any residual irrigant. A sterile paper point was used to obtain the sample from the canal which was deposited in a sterile Eppendorf tube containing 200 µl of BHI broth.

On the basis of concentration of sodium hypochlorite used, this group was further subdivided into two subgroups :-

1A – 2.5% sodium hypochlorite used

1B – 5. 25% sodium hypochlorite used

GROUP 2 – Ultrasonic activated irrigation

It was done using ultrasonic irrigation tips for 1 minute with NaOCl. Then, a paper point was used to take sample from the canal and deposited in a sterile Eppendorf tube containing 200 µl of BHI broth

On the basis of concentration of sodium hypochlorite used, this group was further subdivided into two subgroups :-

2A – 2.5% sodium hypochlorite used

2B – 5. 25% sodium hypochlorite used

GROUP 3 – Laser assisted irrigation

A diode laser of 810 nm at 2 Watts was used with NaOCl irrigant. The fiber tip of 400 microns was inserted into the root canal at a distance of 1 mm from the apical foramen and moved in two consecutive cycles from apical to coronal at a constant speed of approximately 1.5 mm/s. Following this sterile, paper point was used to take sample from the canal and deposited in a sterile Eppendorf tube containing 200 µl of BHI broth.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 1, 2021, Pages. 6227 - 6236
Received 15 December 2020; Accepted 05 January 2021.

On the basis of concentration of sodium hypochlorite used, this group was further subdivided into two subgroups :-
3A – 2.5% sodium hypochlorite used
3B – 5. 25% sodium hypochlorite used
The root canals were sampled immediately before and after the disinfection procedures. After taking the sample from the paper point, blood agar plates were cultured. The BHI agar plates were incubated at 37 degrees for 24 hours. Colony count of E. faecalis was done using semi-quantitative method and expressed in CFU/ml.
Each testing group's antibacterial effectiveness was assessed by observing the number of colonies formed. A high CFU/ml score indicated a lower antibacterial effect.

RESULTS

The results were analysed by counting the colony forming units (CFUs) of Enterococcus faecalis after disinfecting the canals. The evaluation revealed a significant decrease in CFUs in all the three groups.
The results of one-way analysis of variance (ANOVA) showed that mean value of Group-I (223.06), Group-II (7.53) and Group-III (207.24) differ significantly from each other as shown in Table 1 and Figure 1. The comparison of all the subgroups is seen in Table 2 and Figure 2.

Table 1: One-way ANOVA and Bonferroni tests for pair wise inter group comparisons

Groups	Mean±SD	P-value	Interpretation
Group-I	223.06 ± 218.73	< 0.001*	Significant
Group-II	7.53± 8.78		
Group-III	207.24± 204.46		
Pair-wise comparisons (Bonferroni Post-hoc)			
Group-I and Group-II	215.53 (d)	<0.001*	Group-I >Group-II
Group-I and Group-III	15.82 (d)	0.955	Group-I, Group-III
Group-II and Group-III	199.71 (d)	<0.001*	Group-I >Group-II Group-II <Group-I

'd' represents mean difference

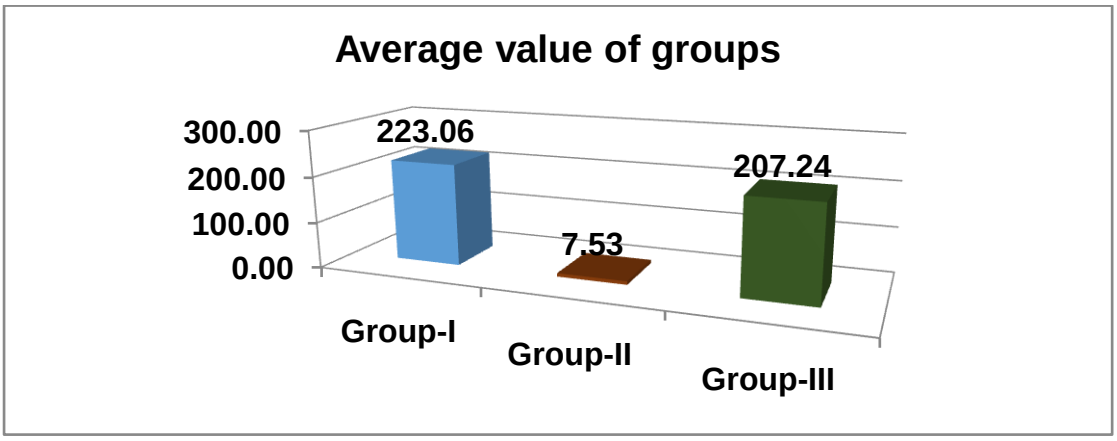

Figure-I: Inter-group comparisons

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 1, 2021, Pages. 6227 - 6236
Received 15 December 2020; Accepted 05 January 2021.

Table 2: One-way ANOVA and Bonferroni tests for pariwise comparisons of sub-groups

Sub-groups	N	Mean±SD	P-value	Interpretation
I A	10	431.78± 64.33	<0.001*	Significant
I B	10	14.34± 7.34		
II A	10	15.06± 6.06		
II B	10	0.00± 0.00		
III A	10	406.08± 19.52		
III B	10	8.40± 3.01		
Pair-wise comparisons of sub-groups(Bonferroni Post-hoc)				
I A and I B	10	d= 417.44	< 0.001*	I A > I B
I A and II A	10	d= 416.72	< 0.001*	I A > II A
I A and II B	10	d= 431.78	< 0.001*	I A > II B
I A and III A	10	d= 25.70	0.647	I A, III A
I A and III B	10	d= 423.38	< 0.001*	I A > III B
I B and II A	10	d= -0.72	1.00	I B, II A
I B and II B	10	d= 14.34	1.00	I B, II B
I B and III A	10	d= 391.74	< 0.001*	I B < III A
I B and III B	10	d= 5.94	1.00	I B, III B
II A and II B	10	d= 15.06	1.00	II A, II B
II A and III A	10	d= 391.02	< 0.001*	II A < III A
II A and III B	10	d= 6.66	1.00	II A, III B
II B and III A	10	d= 406.08	< 0.001*	II B < III A
II B and III B	10	d= 8.40	1.00	II B, III B
III A and III B	10	d= 397.68	< 0.001*	III A > III B

d' represents mean difference

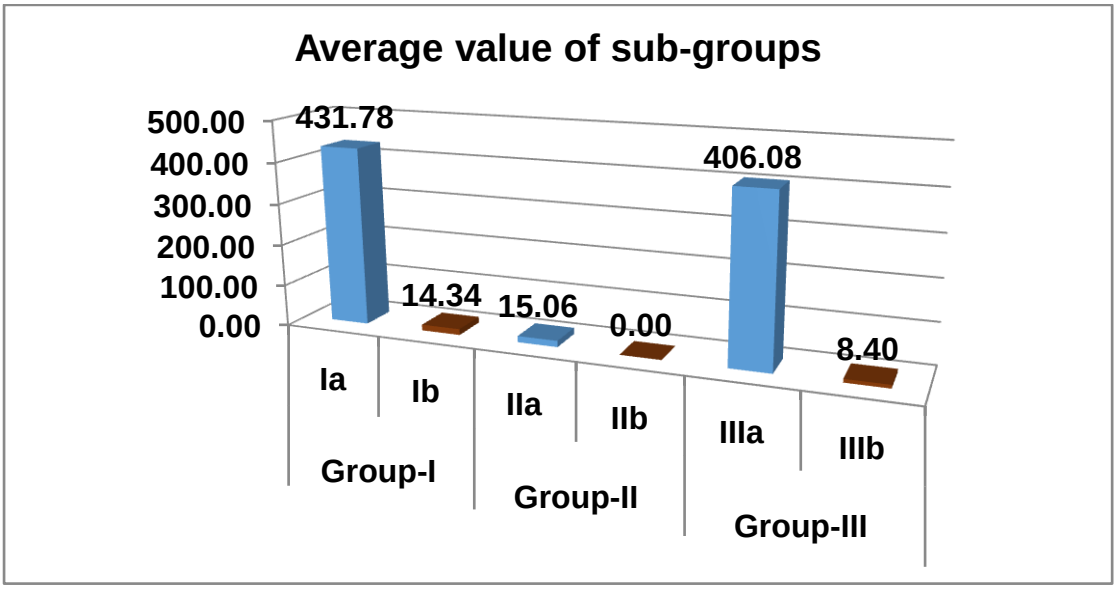

Figure II: Mean value of sub-groups A and B of Group-I, Group-II and Group-III

Irrigation using 2.5% concentration with conventional syringe has shown maximum colony forming units (CFUs – 431.78) of E.fecalis i.e it was least efficacious in eradicating of E fecalis. Using this 2.5% concentration, ultrasonics has shown to be most efficacious (15.06) than lasers (406.08) and conventional syringe irrigation (431.78).

It was seen that on potentiating 2.5% concentration with ultrasonics, the CFUs of E. faecalis (15.06) were statistically similar to the CFU count (14.34)after irrigation with 5.25% concentration using conventional syringe irrigation. CFUs of E. faecalis with 2.5% NaOCl with lasers was significantly more (406.08) than 5.25% using conventional syringe irrigation (14.34).

Irrigation with 5.25% concentration with ultrasonics showed maximum efficacy in eradication of E. faecalis (CFU - 0.00), followed by lasers (CFU - 8.40) and minimum efficacy with conventional syringe irrigation (CFU - 14.34)

DISCUSSION

The effective antibacterial action of sodium hypochlorite is evaluated against Enterococcus faecalis in our study. It has been found to be the most isolated microorganism in refractory endodontic infections and persistent apical periodontitis (Molander A et al, 1998)[14].In an invitro study of 50 teeth that had undergone root canal treatment, Wang et al. (2012)[15]observed an E. fecalis infection in 38% of the 58 teeth with poor root canal treatments that they studied and noted a higher prevalence in teeth with poor obturation.

Antimicrobial effect of sodium hypochlorite increases with increase in concentration and time of exposure. Due to the harmful cytotoxic and caustic properties of more concentrated sodium hypochlorite solutions (Martin et al. 2014)[16], and possibility of apical extrusion of irrigant on increasing the contact time, it will be more desirable to adopt activation of lower concentrations, for the purpose of patient's safety.The efficacy of lower concentrations can be increased by various techniques such as lasers, sonic and ultrasonic irrigation, photodynamic irrigation etc

In thisstudy we investigated whether potentiating the effect of 2.5% NaOCl with passive ultrasonic irrigation and laser achieved an efficacy similar to 5.25%NaOCl with the aim of achieving a better irrigant at low concentration of NaOCl with least irritant effect on periapical tissues.

In our study, we have compared two concentrations of sodium hypochlorite i.e. 5.25% and 2.5%. There was a statistically significant difference between 5.25% and 2.5% concentrations of NaOCl with 5.25% NaOCl performing better against E. fecalis. Similar results were seen by Berber VB et al., 2006[17] and Shehab NF et al., 2019[18] However, few studies did not find any significant difference between these two concentrations (Siqueira et al. 2000[19], Zand et al. 2016[20]).

Among the different irrigation techniques used, ultrasonic irrigation has been found to be more effective in eradicating E fecalis at both the concentrations of NaOCl, in comparison to the diode laser followed by conventional irrigation using syringe.

On comparing ultrasonics and syringe irrigation, there was a significant difference with ultrasonic irrigation being more effective in eradicating E fecalis using both 5.25% and 2.5% NaOCl. This result was supported by Hage W et al. (2019)[21] who found that all the activation techniques (Lasers, ultrasonics and sonics) were superior to conventional syringe irrigation in

eliminating E. fecalis when 5.25% NaOCl was used. Virdee SS et al, (2020)[22] found better tubular penetration of NaOCl with manual dynamic agitation, sonics and ultrasonics as compared to conventional needle irrigation. Also, when 2.5% concentration was used, ultrasonics performed better (Arun J et al, 2017)[10].

The laser light is thought to be able to reach areas that are impossible with the traditional techniques.[23] Among different types of lasers, the diode laser is the most desirable type due to the properties such as high penetration depth into the dentinal tubules and proper antibacterial effect.[24]The antibacterial quality of diode lasers is attributed to the thermal effect and rise in temperature occurring inside the root canals on irradiation.[25]

Diode laser with a wavelength of 810 nm was used in our study because it has shown lowest temperature increases[26] compared with other laser devices and is considered safe for periodontal tissues. Lasers performed significantly better than conventional syringe irrigation at both 5.25% and 2.5% NaOCl. A study by Dai S et al., 2017[27] has shown similar results as that of our study in which diode laser performed better than conventional syringe irrigation when 5.25% NaOCl was used.Similarly, in a study by Thomas S et al., 2017[28], laser group showed significant reduction in the colony count compared to 3% NaOCl irrigation. Mehrvarzfar et al., 2011[29] suggested the combination therapy including chemical irrigation and laser irradiation as an effective treatment option for elimination of E. faecalis from the root canal system.

On comparing lasers and ultrasonics, our study showed significant better disinfection with ultrasonics as compared to laser. Similarly, in a study by Rathod D et al., 2018[30], ultrasonicswith 3% sodium hypochlorite showed 93.68% reduction in E fecalis and lasers showed 82.44% reduction.Contrasting results were seen in a study using 5.25% NaOCl which showed lasers to be more effective than ultrasonics against E fecalis, however the results were not statistically significant. A study by Bago et al., 2014[31], who evaluate the antimicrobial effect of a diode laser irradiation, photo-activated disinfection, conventional and sonic activated irrigation with 2.5% NaOCl against E. faecalis. Their results showed that EndoActivator was more successful in reducing the root canal infection than the diode laser. Another contrasting study by Bago Jurič I[32] showed that Laser, Passive ultrasonic irrigation and RinsEndo were equally effective in the elimination of intracanal 10-day-old E. faecalis when 2.5% NaOCl was used.

Our study also observed that 5.25% NaOCl with conventional syringe irrigation and 2.5% NaOCl with ultrasonic activation performed statistically similar as seen in Table 2.Since a higher cases of post-operative pain is seen with 5.25% NaOCl[33] due to its toxic effects, its effectiveness needs to be compensated using more safer methods. Therefore, lower concentrations of NaOCl should be used ultrasonic agitation to achieve optimum disinfection.

Among the three techniques used, it was seen that conventional needle irrigation was least effective in eradicating E. fecalis. The reason can beattributed to the penetrating depth of irrigant and and its ability to disinfect the root canals is very limited with conventional syringe. The antibacterial effect of lasers can be due to its thermal effects i.e. the rise in intracanal temperature on irradiation. Also, it removes the smear layer that helps in better penetration of irrigants. Passive ultrasonic irrigation, on the other hand has shown better results as it increases the velocity and volume of the irrigant flow within the root canal, thus

making it possible for the irrigant to reach areas of canal irregularities.It increases the tubular penetration of irrigant.

The limitations of our study are that it included only a single species to determine the anti-bacterial effect of E. fecalis. To determine the most effective endodontic protocol, the efficacy of the techniques should be further determined on multispecies biofilm. Also, a larger sample size is required for the confirmation of these results.

CONCLUSION

Enterococcus fecalis species has been implicated from root canals of teeth with posttreatment lesions. NaOCl alone was not effective in eliminating the bacteria completely from the root canals.

With the limitations of this study, it was found that:

1. 5.25% NaOCl was more effective as compared to 2.5% NaOCl in eradicating Enterococcus fecalis.
2. Ultrasonic activated irrigation was more effective at both the concentrations of NaOCl, than lasers and conventional syringe irrigation, in reducing E. fecalis counts, as it disrupt the bacteria's biofilm using both the concentrations.
3. To avoid caustic effects of high concentration of sodium hypochlorite, 2.5% NaOCl assisted with ultrasonics can be used which is as effective as 5.25% NaOCl delivered using syringe.

However, further in vivo studies are required to corroborate the present in vitro study to intra oral conditions.

REFERENCES

1. Love RM. Enterococcus faecalis--a mechanism for its role in endodontic failure. Int Endod J. 2001;34(5):399-405.
2. Zhang, C., Du, J., Peng, Z. Correlation between Enterococcus faecalis and persistent intraradicular infection compared with primary intraradicular infection: a systematic review. (2015) 41(8): 1207-1213.
3. Sundqvist G, Figdor D, Persson S, Sjogren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998;85(1):86-93. 17.
4. Gulabivala K, Ng YL, Gilbertson M, Eames I. The fluid mechanics of root canal irrigation. Physiol Meas. 2010 Dec;31(12):R49-84.
5. Wu MK, van der Sluis LW, Wesselink PR. The capability of two hand instrumentation techniques to remove the inner layer of dentine in oval canals. Int Endod J. 2003;36(3):218–24.
6. Byström A, Sundqvist G. Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. Scand J Dent Res 1981;89:321-8.
7. Sohrabi K, Sooratgar A, Zolfagharnasab K, Kharazifard MJ, Afkhami F. Antibacterial Activity of Diode Laser and Sodium Hypochlorite in Enterococcus Faecalis-Contaminated Root Canals. Iran Endod J. 2016 Winter;11(1):8-12.
8. Zehnder M. Root canal irrigants. J Endod. 2006 May;32(5):389-98.

9. Boutsioukis C, Psimma Z, van der Sluis LW. Factors affecting irrigant extrusion during root canal irrigation: a systematic review. Int Endod J. 2013 Jul;46(7):599-618.
10. Arun J, Shenoy A. Comparative evaluation of efficacy of conventional and passive ultrasonic irrigation with sodium hypochlorite against three endodontic pathogens: An in vitro study. Int J Oral Health Sci 2017;7:86-92.
11. Mostafa MEHAA, El-Shrief YAI, Anous WIO, Hassan MW, Salamah FTA, El Boghdadi RM, El-Bayoumi MAA, Seyam RM, Abd-El-Kader KG, Amin SAW. Postoperative pain following endodontic irrigation using 1.3% versus 5.25% sodium hypochlorite in mandibular molars with necrotic pulps: a randomized double-blind clinical trial. Int Endod J. 2020 Feb;53(2):154-166.
12. Gu LS, Kim JR, Ling J, Choi KK, Pashley DH, Tay FR. Review of contemporary irrigant agitation techniques and devices. J Endod. 2009 Jun;35(6):791-804.
13. Stojicic S, Zivkovic S, Qian W, Zhang H, Haapasalo M. Tissue dissolution by sodium hypochlorite: Effect of concentration, temperature, agitation, and surfactant. J Endod 2010;36(9):1558-1562.
14. Molander A, Reit C, Dahlén G, Kvist T. Microbiological status of root-filled teeth with apical periodontitis. Int Endod J. 1998 Jan;31(1):1-7.
15. Wang QQ, Zhang CF, Chu CH, Zhu XF. Prevalence of Enterococcus faecalis in saliva and filled root canals of teeth associated with apical periodontitis. Int J Oral Sci 2012;4:19-23.
16. Martin DE, De Almeida JF, Henry MA, Khaing ZZ, Schmidt CE, Teixeira FB, Diogenes A. Concentration-dependent effect of sodium hypochlorite on stem cells of apical papilla survival and differentiation. J Endod. 2014 Jan;40(1):51-5.
17. Berber VB, Gomes BP, Sena NT, Vianna ME, Ferraz CC, Zaia AA, Souza-Filho FJ. Efficacy of various concentrations of NaOCl and instrumentation techniques in reducing Enterococcus faecalis within root canals and dentinal tubules. Int Endod J. 2006;39(1):10-7.
18. Shehab NM, Zakaria NA, Taha Mahmoud YM. Efficiency of Sodium Hypochlorite as Root Canal Disinfectant against Enterococcus faecalis: An In Vitro Study. EC Microbiology. 2019; 15(4): 288-94.
19. Siqueira JF Jr, Rôças IN, Favieri A, Lima KC. Chemomechanical reduction of the bacterial population in the root canal after instrumentation and irrigation with 1%, 2.5%, and 5.25% sodium hypochlorite. J Endod. 2000 Jun;26(6):331-4.
20. Zand V, Lotfi M, Soroush MH, Abdollahi AA, Sadeghi M, Mojadadi A. Antibacterial Efficacy of Different Concentrations of Sodium Hypochlorite Gel and Solution on *Enterococcus faecalis* Biofilm. Iran Endod J. 2016 Fall;11(4):315-319.
21. Hage W, De Moor RJG, Hajj D, Sfeir G, Sarkis DK, Zogheib C. Impact of Different Irrigant Agitation Methods on Bacterial Elimination from Infected Root Canals. Dent J (Basel). 2019 Jun 27;7(3):64.
22. Virdee SS, Farnell DJJ, Silva MA, Camilleri J, Cooper PR, Tomson PL. The influence of irrigant activation, concentration and contact time on sodium hypochlorite penetration into root dentine: an ex vivo experiment. Int Endod J. 2020 Jul;53(7):986-997.
23. Odor TM, Watson TF, Pitt Ford TR, McDonald F (1996) Pattern of transmission of laser light in teeth. International Endodontics Journal 29, 228–34.

24. Gutknecht N, van Gogswaardt D, Conrads G, Apel C, Schubert C, Lampert F. Diode laser radiation and its bactericidal effect in root canal wall dentin. J Clin Laser Med Surg. 2000;18(2):57–60.
25. Miserendino L, Robert PM. Hanover Park, IL: Quintessence Publishing; 1995. Lasers in Dentistry.
26. Schoop U, Kluger W, Moritz A, Nedjelik N, Georgopoulos A, Sperr W. Bactericidal effect of different laser systems in the deep layers of dentin. Lasers Surg Med. 2004;35(2):111–6.
27. Dai S, Xiao G, Dong N, Liu F, He S, Guo Q. Bactericidal effect of a diode laser on Enterococcus faecalis in human primary teeth-an in vitro study. BMC Oral Health. 2018 Aug 31;18(1):154.
28. Thomas S, Asokan S, John B, Priya G, Kumar S. Comparison of Antimicrobial Efficacy of Diode Laser, Triphala, and Sodium Hypochlorite in Primary Root Canals: A Randomized Controlled Trial. Int J Clin Pediatr Dent 2017;10(1):14-17.
29. Mehrvarzfar P, Saghiri MA, Asatourian A, Fekrazad R, Karamifar K, Eslami G, Dadresanfar B. Additive effect of a diode laser on the antibacterial activity of 2.5% NaOCl, 2% CHX and MTAD against Enterococcus faecalis contaminating root canals: an in vitro study. J Oral Sci. 2011;53(3):355-60.
30. Rathod D, Mulay S. Evaluation of efficacy of Nd:YAG laser, ozonated water, and ultrasonic irrigation with 3% sodium hypochlorite for disinfection of root canal: An in vivo study. Endodontology 2018;30:135-9.
31. Bago Jurič I, Plečko V, Anić I. Antimicrobial efficacy of Er,Cr:YSGG laser-activated irrigation compared with passive ultrasonic irrigation and RinsEndo(®) against intracanal Enterococcus faecalis. Photomed Laser Surg. 2014 Nov;32(11):600-5.
32. Bago I, Plečko V, Gabrić Pandurić D, Schauperl Z, Baraba A, Anić I. Antimicrobial efficacy of a high-power diode laser, photo-activated disinfection, conventional and sonic activated irrigation during root canal treatment. Int Endod J. 2013 Apr;46(4):339-47.
33. Mathew J, Emil J, Paulaian B, John B, Raja J, Mathew J. Viability and antibacterial efficacy of four root canal disinfection techniques evaluated using confocal laser scanning microscopy. J Conserv Dent. 2014 Sep;17(5):444-8.